⑫ **EUROPEAN PATENT APPLICATION**

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�54 Receptors of Interleukin-12 and antibodies.

㊄ This invention relates to Interleukin-12 receptor cDNAs, proteins and uses thereof.

Fig. 2

This inv ntion relat s generally to cytokin receptors and mor specifically relates to Interleukin-12 r ceptors.

In ord r for a molecule such as IL-12 to x rt its effect on c lls, it is now acc pted by thos skilled in th art that th molecul must interact with molecules, located on c ll membran s, r f rred to as rec ptors.

5 Patents which exemplify disclosures of interleukin receptors include Honjo et al., U.S. Patent No. 4,816,565; Urdal et al., U.S. Patent No. 4,578,335; Dower et al., U.S. Patent No. 5,180,812; and Taniguchi et al., U.S. Patent No. 5,198,359.

Also soluble forms of interleukin receptors are known (Fanslow, W.C. et al. (1990) Science 248, 739-41). The effect of Interleukin-1 (IL-1) *in vivo* could be regulated via the administration of a soluble form of its
10 receptor. The results that Fanslow report demonstrate the ability of a soluble cytokine receptor (soluble IL-1R) to modulate biological activity upon exogeneous administration *in vivo*, presumably by acting as a neutralizing agent for the endogeneously produced, corresponding ligand (IL-1), and provides evidence of the therapeutic potential of soluble cytokine receptors in a variety of clinical disorders. Systemic administration of a soluble, extracellular portion of the receptor for IL-1 (soluble IL-1R) had profound inhibitory effects
15 on the development of *in vivo* alloreactivity. Survival of heterotopic heart allografts was prolonged from 12 days in controls to 17 days in mice treated with soluble IL-1R. Lymph node hyperplasia in response to localized injection of allogeneic cells was completely blocked by soluble IL-1R treatment.

Interleukin-12 (IL-12), formerly known as cytotoxic lymphocyte maturation factor or natural killer cell stimulatory factor, is a 75-KDa heterodimeric cytokine composed of disulfide-bonded 40-KDa (p40) and 35-
20 KDa (p35) subunits that has pleiotropic activities including stimulation of the proliferation of activated T and NK cells (Gately, M. K., et al. (1991) J. Immunol., 147, 874) (Kobayashi, M. et al. (1989) J. Exp. Med., 170, 827), enhancement of the lytic activity of NK/LAK cells (Kobayashi, M., et al., supra; Stern, A.S., et al. (1990) Proc. Natl. Acad. Sci. USA, 87, 6808), enhancement of cytolytic T-cell responses (M. Gately et al. (1992) Cell. Immunology, 143, 127), induction of interferon gamma by resting and activated T-and NK-cells
25 (M. Kobayashi et al., supra; S. H. Chan et al. (1991) J. Exp. Med., 173, 869), and promotion of T_h1-type helper cell responses (R. Manetti et al. (1993) J. Exp. Med., 177, 1199; C.-S. Hsieh et al. (1993) Science 260, 547).

The biological activity of IL-12 is mediated by the binding of the IL-12 molecules to cell surface, or plasma membrane, receptors on activated T- and NK cells; however, the contributions of the individual
30 subunits, p35 and p40, to receptor binding and signal transduction remain unknown. Studies with labelled IL-12 have shown that this binding occurs in a specific and saturable manner. IL-12 delivers a signal to target cells through a receptor that was initially characterized on PHA-activated CD4+ and CD8+ T-cells and on IL-2 activated CD56+ NK-cells (R. Chizzonite et al. (1992) J. Immunol., 148, 3117; B. Desai, et al. (1992) J. Immunol., 148, 3125). A survey of over 20 human cell lines belonging to the T-, B-, NK- and
35 myelomonocytic lineages only identified a single CD4+, IL-2 dependent human T-cell line (Kit 225) that constitutively expresses the IL-12 receptor and responds to IL-12 (B. Desai, et al. (1992) J. Immunol., 148, 3125; B. Desai, et al. (1993) J. Immunol. 150, 207A). Freshly prepared PHA-activated PBMC and the Kit 225 cell line thus represent two convenient cell sources to study the biochemistry of the functional IL-12 receptor; there may be others. Equilibrium binding experiments with ^{125}I-labelled IL-12 showed that i) PHA-
40 activated PBMC express several thousand IL-12 receptors which show 3 classes of affinities: high = 5-20 pM, intermediate = 50-200 pM and low = 2-6 nM; ii) IL-12 receptor expression on PBMC is upregulated by mitogen or IL-2 stimulation; and iii) the IL-12 receptor upregulation correlates with the ability of the cells to proliferate in response to IL-12 (R. Chizzonite, et al. (1992) J. Immunol., 148, 3117; B. Desai, et al. (1992) J. Immunol., 148, 3125). It was not clear at this point whether the biologically functional IL-12 receptor consists
45 of one or more subunits. Affinity crosslinking of labelled IL-12 to activated PBMC demonstrated the size of the cell surface IL-12 binding protein(s) under nonreducing conditions to be in the range of about 150 KDa to about 200 KDa. Additional affinity crosslinking and immunoprecipitation experiments with unlevelled IL-12 bound to ^{125}I-surface labelled activated PBMC identified an IL-12 binding protein that under reducing conditions had a size of about 110 KDa (R. Chizzonite, et al. (1992) I. Immunol., 148, 3117).

50 The present invention is directed towards an isolated cDNA encoding a human low affinity IL-12 receptor protein or subunit thereof. When expressed in mammalian cells, the cDNA gives rise to substantially homogeneous IL-12 receptor protein that binds IL-12 in a specific and saturable manner with an apparent affinity of about 2-10 nM, preferably 2-5 nM. Further, this inv ntion relates to novel antibodies against th interleukin-12 rec ptor. R pres ntativ anti-int rl ukin-12 antis ra provided in accordanc with
55 th pr s nt inv ntion block interl ukin-12 binding to cells expressing interleukin-12 rec ptors, and can also neutraliz interl ukin-12 activity.

Brief d scription of th drawings:

Figur 1: DNA s qu nce of human IL-12 receptor cDNA clone No. 5. (translat d portion = nucleotides 65 to 2050) (SEQ ID NO:1).

5   Figure 2: Amino acid sequence of human IL-12 receptor protein as deduced from cDNA sequence of Figure 1. (underlined amino acid residues of N-terminal sequence = signal peptide sequence; amino acid residues nos. 541 to 571 = transmembrane area marked by ——; 6 potential N-linked glycosylation sites in the extracellular portion marked by ———; conserved areas 1 and 2 of the cytoplasmic domain are marked by

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[amino acid residues nos. 577 to 584 and 618 to 629] (SEQ ID NO:2).

15   Figure 3: Alignment of the IL-12 receptor protein subunit sequence with human gp130, human granulocyte colony-stimulating factor-receptor (G-CSF-R) and leukemia inhibitory factor-receptor (LIF-R), and resulting consensus sequence. Consensus residues indicated by lowercase letters refer to identities between IL-12 receptor and gp130 only. The following sequence ranges were used: IL-12 receptor protein (SEQ ID NO:2): residues 42-662; gp130: residues 124-742 (Hibi et al. (1990) Cell, 63, 1149); G-

20   CSF-R: residues 98-731 (Fukunaga, et al. (1990) Proc. Natl. Acad. Sci. USA, 87, 8702); LIF-R: residues 331-950 (Gearing, et al. (1991) EMBO J., 10, 2839).

Figure 4A: Scatchard analysis of IL-12 binding to recombinant human IL-12 receptor expressed in COS cells.

Figure 4B: Scatchard analysis of 2-4E6 antibody binding to recombinant human IL-12 receptor expressed

25   in COS cells.

Figure 5: Analysis of the size of the IL-12 receptor protein under reducing conditions. COS cells were transfected with the IL-12 receptor cDNA and labelled with ^{35}S cysteine as described. Aliquots of cell lysates were immunoprecipitated with different antibodies as follows: Lane 1: negative control Ig; lane 2: 2-4E6 antibody; lane 3: negative isotype matched control antibody 4D6. Marker sizes in KDa are

30   indicated on the left of the figure.

Figure 6: Analysis of the sizes of IL-12 receptor transcripts by RNA blotting. 4 µg of poly A+ RNA were run per lane. Lanes 1-4 and 5-8 represent identical loadings. Lanes 1-4 were probed with a full length cDNA to the receptor, lanes 5-8 with a probe corresponding only to the cytoplasmic portion of the receptor. Lanes 1, 5: unstimulated peripheral blood mononuclear cells (PBMC). Lanes 2, 3, 6, 7: PHA

35   stimulated PBMC. Lanes 1 and 6 represent a 3 day induction of the sample shown in lanes 1 and 5. Samples in lanes 3 and 7 are from an independent 4 day PHA induction and lanes 4 and 8 represent RNA from K6 cells. The bottom panel shows rehybridization of the blots with a probe with a probe for ribosomal protein L32 (loading control).

Figure 7: Inhibition of ^{125}I-IL-12 binding to IL-12 (IL-12R) receptor by mouse anti-IL-12R antiserum

40   Figure 8: Characterization of the IL-12 binding proteins on IL-12R positive human cells by affinity-crosslinking

Figure 9: Immunoprecipitation of the solubilized ^{125}I-IL-12/IL-12R crosslinked complex by anti-IL-12R antibodies

Figure 10: Equilibrium binding of ^{125}I-2-4E6 to PHA-activated PBMC at room temperature

45   Figure 11: Equilibrium binding of ^{125}I-2-4E6 to human K6 cells at room temperature

Figure 12: Inhibition of ^{125}I-2-4E binding to K6 cells by purified 2-4E6 (24E6), human IL-12 (HUIL-12) and control antibody (control IgG)

Figure 13: Equilibrium binding of ^{125}I-IL-12 to human K6 cells at room temperature

Figure 14: Equilibrium binding of ^{125}I-IL-12 to detergent solubilized IL-12R from K6 cells

50   Figure 15: Western blot analysis of detergent solubilized IL-12R with mAb 2-4E6

Figure 16: Detection of IL-12 receptor on human cells by flow cytometry

Figure 17: Size of the IL-12 receptor subunit on the surface of transfected COS and CTLL cells. 8% gels were used and marker siz s in KDa are indicated. Lanes 1-4: Analysis of affinity crosslinked complexes und r non-r ducing conditions. Arrowh ad = labelled, uncrosslinked 2-4E6 antibody. Arrows = labell d,

55   uncrosslinked IL-12. Lanes 5-12: Analysis of ^{125}I-COS Cell Surfac Prot ins. Sampl reduction, binding of 25 nM unlabelled IL-12 to the c lls prior to analysis and us of 1 mM DTSSP crosslinker are indicated below the lan s. Lan s 13-16: Analysis of ^{125}I-CTLL c ll surfac prot ins.

Figur 18: Specific inhibition of IL-12-induc d lymphoblast prolif ration by a rat anti-IL-12R antis rum. (A) Titration by flow cytom try of anti-COS c II antibodies in an anti-IL-12R antiserum (--•--) made against 2-4E6-transfected COS c lls, preimmun serum (--o--) from th rat used to pr par the anti-IL-12R antiserum, and a rat antiserum mad against COS c lls transfect d with th human typ II IL-1R (-- ■ --). (B-D) Effects of rat sera on proliferation of PHA-activated PBMC induced by IL-12 (B), IL-2 (C), or IL-7 (D). All standard errors were <10% of the means.

Figure 19: Equilibrium binding of ^{125}I-IL-12 to COS cells expressing the IL-12 receptor subunit. A) human IL-12 and B) murine IL-12. The insets show analysis of the binding data according to the method of Scatchard.

The present invention is directed to the low affinity receptor of Interleukin-12 (IL-12). The invention includes the homogenous natural IL-12 receptor including allelic variants and subunits as well as functional derivatives thereof: proteins containing a fragment of the natural IL-12 receptor with binding activity for IL-12. Furthermore the present invention includes recombinant IL- 12 receptor proteins as well as fusion proteins, i.e. IL-12 receptor derivatives comprising the amino acid sequence of the natural IL-12 receptor or a partial sequence thereof together with amino acid sequences derived from other proteins. The proteins or polypeptides of the present invention have binding activity for IL-12 as measured by standard assays as described below in the Examples.

A preferred aspect of the present invention relates to a human low affinity IL-12 receptor protein or subunit thereof, more preferred the human receptor protein with the amino acid sequence SEQ ID NO:2 or SEQ ID NO:3.

The invention also includes a soluble form of the IL-12 receptor, preferably a protein exhibiting having IL-12 binding activity, said protein having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:3 or a portion thereof which encodes a truncated IL-12 receptor protein. Soluble forms of the IL-12 receptors can be prepared by methods known in the art: by isolating soluble IL-12 receptor proteins from culture medium, of IL-12 receptor expressing cells chemical or enzymatic cleavage of IL-12 receptor protein or by recombinant DNA technology.

The availability of the purified receptor, preferably in soluble form, presents therapeutic possibilities as well. Addition of soluble IL-12 receptor interferes with the effect of the interleukin on the cells, since the molecule cannot bind to the cell membrane as freely. Hence, an aspect of the invention is the treatment of pathological conditions caused by excess activity of cells possessing IL-12 receptors by adding an amount of soluble IL-12 receptors sufficient to inhibit binding of IL-12 to the aforementioned cells. This methodology can also be modified, and the soluble receptor can be used as a screening agent for pharmaceuticals. Briefly, a pharmaceutical which works as an IL-12 antagonist can do so by blocking the binding of IL-12 to the IL-12 receptor. Prior to determining whether a material would be effective *in vivo*, one may use the purified IL-12 receptor in connection with a potential pharmaceutical to determine if there is binding. If not, then the pharmaceutical may no longer be a desirable candidate. If there is in fact binding, further testing may be indicated.

The IL-12 receptor proteins of the present invention also include non-naturally occurring IL-12 receptor analogous proteins or mutants. These kind of functional derivatives are proteins in which one or more of the amino acids of the natural IL-12 receptor or its fragments have been replaced or deleted without loss of the IL-12 receptor binding activity. Such analogues may be produced by known methods of peptide chemistry or by recombinant DNA technology.

The term IL-12 receptor protein comprises also derivatives which may be prepared from the functional groups occurring as side chains on the residues or the N- or C-terminal groups, by means known in the art, and are included in the invention as long as they remain pharmaceutically acceptable, i.e. they do not destroy the activity of the protein and do not confer toxic properties on compositions containing it. These derivatives may include, for example, polyethylene glycol side-chains which may mask antigenic sites and extend the residence of the Interleukin-12 receptor protein in body fluids. Other derivatives include aliphatic esters of the carboxyl groups, amides of the carboxyl groups by reaction with ammonia or with primary or secondary amines, N-acyl derivatives of free amino groups of the amino acid residues formed with acyl moieties (e.g. alkanoyl or carbocyclic aroyl groups) or O-acyl derivatives of free hydroxyl groups (for example that of seryl- or threonyl residues) formed with acyl moieties.

Th present inv ntion also relates to cloned DNA sequ nces coding for the IL-12 receptor and to polynucleotid s ncoding a prot in as d fined abov , which polynucleotide contains a sequence cor-responding to th cDNA encoding th IL-12 rec ptor, to recombinant vectors comprising a polynucleotide encoding an IL-12 receptor protein, to microorganisms transform d with said recombinant vectors as well as to proc sses for the pr paration of said proteins, polynucleotid s and v ctors.

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The t rm "polynucl otide containing a sequenc corr sponding to th cDNA encoding th IL-12 rec ptor" refers to a polynucleotid containing a sequ nce which is homologous to or compl mentary to a sequenc in th sequ nc encoding the IL-12 rec ptor. Th d gree of homology to th cDNA will b at least about 80%, pref rably at least about 90%.

5 The practice of the present invention will employ, unless otherwise indicated conventional techniques of molecular biology, microbiology, recombinant DNA and immunology, which are within the skills of an artisan in the field. Such techniques are explained fully in the literature. See e.g. Sambrook, Fritsch & Maniatis, Molecular Cloning; a laboratory Manual (1989) Cold Spring Harbor Laboratory Press 1989.

The DNA sequences and DNA molecules of the present invention may be expressed using a wide 10 variety of host vector combinations. For example, useful expression vectors may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Examples of such vectors are viral vectors, such as the various known derivatives of SV40, bacterial vectors, such as plasmids from *E. coli*, phage DNAs, such as the numerous derivatives of phage λ. M13 and other filamentous single-stranded DNA phages, as well as vectors useful in yeasts, such as the 2μ plasmid, vectors useful in eukaryotic cells more 15 preferably vectors useful in animal cells, such as those containing SV40, adenovirus and/or retrovirus derived DNA sequences.

An aspect of the present invention is a process for preparing a DNA molecule coding for the low affinity Interleukin-12 receptor as described above, characterized in a) the preparation of an anti-Interleukin-12 receptor antibody, b) screening of expression products of a cDNA library, with said antibody, c) identifying 20 and isolating the Interleukin-12 receptor cDNA and d) if desired, preparing a DNA molecule which codes for a functional derivative. A further aspect of the present invention is a process for preparing a low affinity Interleukin-12 receptor protein as described above, characterized in that a) a cell is transformed with a vector comprising a clones gene coding for a low affinity Interleukin-12 receptor protein, b) expression of said protein, c) recovering of said protein and, if desired converting it into a functional derivative thereof.

25 As used herein, "DNA sequence" refers to a DNA polymer, in the form of a separate fragment or as a component of a larger DNA construct, which has been derived from DNA isolated at least once in substantially pure form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide se- quences by standard biochemical methods, for example, using a cloning vector. Such sequences are 30 preferably provided in the form of an open reading frame uninterrupted by internal nontranslated se- quences, or introns, which are typically present in eukaryotic genes. However, it will be evident that genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

35 As used herein, "expression vector" refers to a plasmid comprising a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in various eukaryotic expression systems preferably include a leader sequence 40 enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

The host cell used for the expression of DNA sequence may be selected from a variety of known hosts. 45 Examples for such hosts are prokaryotic or eukaryotic cells. A large number of such hosts are available from various depositories such as the American Type Culture Collection (ATCC) or the Deutsche Sammlung fur Mikro-organismen (DSM). Examples for prokaryotic cellular hosts are bacterial strains such as *E. coli, B. subtilits* and others. Preferred hosts are mammalian cells such as the SV40 transformed African Green monkey kidney cell line COS.

50 The IL-12 receptor protein produced by fermentation of the prokaryotic and eukaryotic hosts trans- formed with the DNA sequences of this invention can then be purified to essential homogeneity by known methods such as, for example, by centrifugation at different velocities, by precipitation with ammonium sulphat , by dialysis (at normal pressure or at reduced pr ssur), by pr parative isoelectric focusing, by pr parative g l lectrophor sis or by various chromatographic methods such as gel filtration, high perfor- 55 manc liquid chromatography (HPLC), ion xchang chromatography, r v rs phase chromatography and affinity chromatography (.g. on Sepharose™ Blue CL-6B or on carrier-bound monoclonal antibodies).

Th siz of th IL-12 r c ptor subunit at th cell surface was stimated by affinity crosslinking of labelled IL-12 as well as cell-surfac labelling studies. Transfected COS cells expr ss th IL-12 receptor

subunit as a protein of about 100 KDa siz . The calculated mol cular weight for th matur form of th prot in is 70,426; thus, about 25% of the molecular weight of the surface expressed protein is likely to be carbohydrat . Transf cted COS cells also xpress a larg r molecular w ight form of th IL-12 rec ptor subunit.

5   The available evidence supports the conclusion that the IL-12 receptor dimerization/oligomerization is independent of IL-12 binding. Similar to these findings, it has been reported for the EPO receptor that disulfide-bonded receptor dimers and oligomers are formed and that EPO stimulation had no detectable effect on receptor dimerization (O. Miura, et al. (1993) Archives Biochem. Biophys., 306, 200). Our data also indicate that only the IL-12 receptor dimers/oligomers bind IL-12 with the 2-5 nM affinity observed on intact
10   transfected COS cells. i) An anti-IL-12 antibody only immunoprecipitates an affinity crosslinked complex corresponding to one IL-12 and a receptor dimer/oligomer. ii) Affinity crosslinked complexes of the size expected for one receptor subunit and one IL-12 are formed very inefficiently at IL-12 concentrations corresponding to the K_D measured on transfected COS cells. iii) Murine CTLL cells stably expressing the receptor subunit bind IL-12 very inefficiently (estimated K_D = 50 nM or lower); these cells also do not
15   express subunit dimers/oligomers. It was unexpected to find that COS cells and CTLL cells differ in their ability to express the IL-12 receptor subunit in a way that allows IL-12 binding. This could be due to species specificity: murine CTLL cells are somehow unable to "process" the human IL-12 receptor protein correctly, resulting in inefficient dimerization/oligomerization and IL-12 binding. Conceivably, COS cells could express an endogenous protein that allows the IL-12 receptor dimerization/oligomerization to occur. Since under the
20   experimental conditions used, the number of low-affinity IL-12 receptor sites per transfected COS cell is always greater than 10^5, it seems unlikely that an endogenous COS cell component forms dimers or oligomers with the receptor subunit.

The IL-12 receptor subunit is a member of the hemopoietin receptor superfamily. Within that family, it is most closely related over its entire length to gp130 and the receptors for G-CSF and LIF. The extracellular
25   portion of the IL-12 receptor subunit can also be divided into five fibronectin type III repeats, similar to what was reported for gp130 (M. Hibi, et al. (1990) Cell, 63, 1149). It is interesting to note that the ligands for IL-12 receptor and gp130, i.e. IL-12 p40 and IL-6 receptor, both also contain such fibronectin type III repeats (Hibi, et al., supra; Schoenhaut, et al. (1992) J. Immunol., 148, 3433). Some features of the cytoplasmic portion of the IL-12 receptor subunit merit further comment. Compared to the corresponding areas in gp130
30   (276 AA) and the receptor for LIF (237 AA), it is rather short. However, mutagenesis studies have shown that for gp130, only about 100 amino acids in the cytoplasmic region are sufficient to transduce a signal (Murakami, et al. (1991) Proc. Natl. Acad. Sci. USA, 88, 11349). The potential functionality of the IL-12 receptor cytoplasmic portion appears to be borne out further by the presence of a number of features conserved in other functional hemopoietic receptors (among them the receptors for G-CSF, EPO and GM-
35   CSF): conserved areas 1 and 2 (Murakami, et al. (1991) Proc. Natl. Acad. Sci. USA, 88, 11349) are clearly present and thus give the low affinity IL-12 receptor component the makeup of a beta type subunit (N. Stahl, et al., 1993, Cell, 74:587).

The IL-12 receptor cDNA is useful for the following purposes:

Expression of recombinant IL-12 receptor protein in high levels and its use as an antigen allows
40   production of additional neutralizing monoclonal and polyclonal antibodies. Such neutralizing antibodies can be used in *in vivo* model settings to elucidate the role that IL-12 and its receptor play in normal as well as pathologic immune responses (i.e. disease states that are aggravated by activated T- and NK-cells like autoimmune diseases, graft versus host disease and rheumatoid arthritis).

IL-12 receptor proteins can be administered, for example, for the purpose of suppressing immune
45   responses in a human. A variety of diseases or conditions are caused by an immune response to alloantigen, including allograft rejection and graft-versus-host reaction. In alloantigen-induced immune responses, IL-12 receptor can suppress lymphoproliferation and inflammation which result upon activation of T cells. IL-12 receptor can therefore be used to effectively suppress alloantigen-induced immune responses in the clinical treatment of, for example, rejection of allografts (such as skin, kidney, and heart transplants),
50   and graft-versus-host reactions in patients who have received bone marrow transplants.

IL-12 receptor can also be used in clinical treatment of autoimmune dysfunctions, such a rheumatoid arthritis, diabetes and multiple sclerosis, which are dependent upon the activation of T cells against antigens not recognized as being indig nous to th host. IL-12 receptor can also b us ful in treatment of septic shock in which int rf ron gamma produced in response to IL-12 plays a central rol in causing morbidity
55   and mortality (Doherty t al. (1992) J. Immunol. 149, 1666).

Purifi d IL-12 rec ptor compositions will be useful in diagnostic assays for IL-12 or IL-12 rec ptor, and also in raising antibodi s to IL-12 rec ptor for use in diagnosis or th rapy. In addition, purifi d IL-12 r c ptor compositions may b used dir ctly in th rapy to bind or scav ng IL-12, th reby providing a

m ans for regulating the immune or inflammatory activities of IL-12. In its us to prevent or r verse pathologic immune responses, soluble IL-12 rec ptor can be combined with other cytokine rec ptor antagonists such as antibodies to th IL-2 r c ptor, soluble TNF receptor, the IL-1 rec ptor antagonist, and the like.

5   The dose ranges for the administration of the IL-12 receptor proteins and fragments thereof may be determined by those of ordinary skill in the art without undue experimentation. In general, appropriate dosages are those which are large enough to produce the desired effect, for example, blocking the binding of endogenous IL-12 to its natural receptor. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will
10 vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. The expected dose range is about 1 ng/kg/day to about 10 mg/kg/day. The IL-12 receptor proteins and fragments thereof can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

15 Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated
20 Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, anti-microbials, anti-oxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 18th Ed., Mack Eds., 1990.

The present invention also relates to novel immunoglobulins, which include novel antisera and
25 monoclonal antibodies to the human IL-12 receptor for all three classes of affinities mentioned above.

Representative anti-IL-12R antisera provided in accordance with the present invention block IL-12 binding to cells expressing IL-12R and can also neutralize IL-12 activity. In further embodiments of the present invention, the monoclonal antibodies which are selective to IL-12R are prepared in accordance with generally known techniques, such as the method of Köhler and Milstein. Suitable monoclonal antibodies to
30 IL-12R can be modified by known methods to provide chimeric, humanized or single chain antibody (SCA) embodiments.

The immunoglobulins according to the present invention comprise antibodies which are capable of inhibiting the binding of IL-12 to the IL-12 receptor and are capable of neutralizing IL-12 bioactivity by binding to the IL-12 receptor. Further the invention refers to immunoglobulins which bind to the IL-12
35 receptor but which are not capable of inhibiting the binding of IL-12 to the IL-12 receptor and are not capable of neutralizing IL-12 bioactivity by binding to the IL-12 receptor.

The IL-12 receptor antibodies of the present invention can be used to determine IL-12 receptor expression on human cells, such as peripheral blood lymphocytes and bone marrow cells, in normal and pathological conditions. The antisera and monoclonal antibodies of the invention can also be used to block
40 IL-12 binding to its receptor and thus block its biologic activity. Neutralizing antibodies of the present invention can thus be used for therapeutic intervention in a number of disease states that are aggravated by activated T-cells and NK cells, such as autoimmune diseases, graft versus host disease and rheumatoid arthritis. Finally, as has been specifically demonstrated by the specific antibody embodiment of the present invention, such antibody will also be useful for expression cloning strategies to isolate a cDNA coding for
45 the IL-12 receptor.

The antisera of the invention can be conveniently produced by immunizing host animals with PHA-activated human PBMC. Suitable host animals include rodents, such as, for example, mice, rats, rabbits, guinea pigs and the like, or higher mammals such as goats, sheep, horses and the like. Initial doses and booster shots can be given according to accepted protocols for eliciting immune responses in animals, e.g.,
50 in a preferred embodiment mice received an initial dose of 6×10^7 cells/mouse i.p. and five subsequent booster shots of between $2-5 \times 10^7$ cells over a six month period. Immunized mice were observed to develop an immune response against the human IL-12R as determined by inhibition of ^{125}I-IL-12 binding to PHA-activated PBMCs (Figure 7) and immunoprecipitation of th complex of ^{125}I-IL-12 crosslinked to IL-12R, which methods provid a conveni nt way to screen for hosts which ar producing antisera having th
55 d sired activity.

Monoclonal antibodies are produced conv ni ntly by immunizing Balb/c mic according to the above sch dul follow d by injecting the mic with 1×10^7 cells i.p. and 2.5×10^6 c lls iv. on two succ ssive days starting four days prior to th cell fusion. Other protocols w ll known in th antibody art may of cours be

utilized as well. The complete immunization protocol detail d her in provid d an optimum protocol for serum antibody respons for the antibody to the human IL-12 rec ptor. Oth r immunization protocols r sulted in a low r s rum antibody response than th protocol s t forth b low: For exampl , 1) immunization with lower numbers of PHA-activat d lymphoblasts (0.7 to 1.8 x 10^7 c lls/booster immunization); 2) immunization with fewer numbers of booster immunization or over a short period of time (40 days) with 2 to 6 x 10^7 cells/immunization; and 3) immunizations with cell membranes derived from PHA-activated lymphoblasts (membranes equivalent to 1 to 4 x 10^8 cells/immunization) all produced serum antibody responses but not as significant as the protocol below. Similar results were obtained when immunizing rats.

B lymphocytes obtained from the spleen, peripheral blood, lymph nodes or other tissue of the host may be used as the monoclonal antibody producing cell. Most preferred are B lymphocytes obtained from the spleen. Hybridomas capable of generating the desired monoclonal antibodies of the invention are obtained by fusing such B lymphocytes with an immortal cell line, that is a cell line that which imparts long term tissue culture stability on the hybrid cell. In the preferred embodiment of the invention the immortal cell may be a lymphoblastoid cell or a plasmacytoma cell such as a myeloma cell, itself an antibody producing cell but also malignant. Murine hybridomas which produce IL-12R monoclonal antibodies are formed by the fusion of mouse myeloma cells and spleen cells from mice immunized against hIL-12R expressed on the surface of activated peripheral blood mononuclear cells. Chimeric and humanized monoclonal antibodies can be produced by cloning the antibody expressing genes from the hybridoma cells and employing recombinant DNA methods now well known in the art to either join the subsequence of the mouse variable region to human constant regions or to combine human framework regions with complementarity determining regions (CDR's) from a donor mouse or rat immunoglobulin, EP 0239400). An improved method for carrying out humanization of murine monoclonal antibodies which provides antibodies of enhanced affinities is set forth in International Patent Application No. WO 92/11018.

The invention also includes a process for the preparation of a monoclonal antibody directed to an Interleukin-12 receptor which comprises a) immunizing a host animal with Interleukin-12 receptor, b) fusing B lymphocytes of said host animal with an immortal cell line, and c) preparation of monoclonal antibodies by culturing the resulting hybridoma cell line.

Polypeptide fragments comprising only a portion of the primary antibody structure may be produced, which fragments possess one or more immunoglobulin activities. These polypeptide fragments may be produced by proteolytic cleavage of intact antibodies by methods well known in the art, or by inserting stop codons at the desired locations in expression vectors containing the antibody genes using site-directed mutagenesis to produce Fab fragments or (Fab')₂ fragments. Single chain antibodies may be produced by joining VL and VH regions with a DNA linker (see Huston et. al., Proc. Natl. Acad. Sci. U.S.A., 85, 5879-5883 (1988) and Bird et. al., Science, 242, 423-426 (1988).

It is also within the skill of the art to utilize the monoclonal antibodies of the present invention as therapeutic agents. They may be formulated for parenteral administration in a manner known in the art such as by dissolving the purified monoclonal antibody product either intact or as a fragment in water for injection and sterile filtering. The dosage form may contain known excipients for parenteral administration of proteins such as buffers, stabilizers and carrier protein. The administered dosage will be selected by the attending physician by giving due consideration to the disease severity and nature as well as the age, size and condition of the patient. As immunoglobulins have demonstrated extended half-lifes in patients dosing every 10-14 days is usually sufficient. It is also within the skill of the art to modify the monoclonal antibody by forming a hybrid with a toxin molecule such as with a pseudomonas exotoxin or with the A chain of ricin to provide a hybrid molecule capable of destroying the cells expressing the IL-12R in a selective manner.

The invention also pertains to a method for detecting peripheral blood cells which express the IL-12 receptor, which comprises contacting a sample which contains the subject cells with substances capable of forming complexes with the IL-12 receptors so as to form cellular complexes between the substances and the IL-12 receptors, and detecting such cellular complexes. Another embodiment of the invention provides a method of evaluating cell activity in a subject which comprises detecting peripheral blood cells according to the method described above.

In the preferred embodiments, the substances are capable of forming complexes only with the IL-12 receptors present on the surface of peripheral blood cells in which the receptors were expressed. Particularly pr f rred ar substanc s which comprise IL-12 monoclonal antibody.

On mbodim nt of th inv ntion provid s a m thod of valuating immun c llular activity which comprises:

    a. isolating peripheral blood mononucl ar c lls;

    b. treating the c lls with th IL-12 monoclonal antibody; and

    c. det rmining the amount of monoclonal antibody bound to th c lls.

Th invention also involv s a m thod for diagnosing an immun syst m abnormality in a subject which compris s determining the number of T cells, NK cells, or B-cells in a sampl d rived from the subject. This method involves contacting the sample with substances capabl of forming compl x s with th IL-12 r ceptors and d t rmining th perc ntag of T c lls, NK c lls or B c lls in th sampl which have th IL-12
5   receptor. Comparing the percentages so determined with the percentage of cells which have the IL-12 receptor in a sample from a normal subject who does not have the immune system abnormality, the differences in the percentage of cells so determined being indicative of the immune system abnormality. Preferably, the subject is an animal, e.g., a human.

As a molecule associated with T cell, NK cell and B cell function, the measurement of IL-12R
10   expression has diagnostic importance. Because IL-12R is distinctive to activated T cells, NK cells or B cells, it is a unique marker for these cells in a population of lymphocytes.

Moreover, the level of expression of IL-12R provides a measure of T cell, NK cell or B cell activity. This information may be important for evaluating the immune status of an individual. For instance, in the treating of certain disease, such as cancer, agents which affect the immunocompetency are often used. Assays for
15   IL-12R expression may allow physicians to monitor the immune status of the patient and to adjust treatment to minimize the risk of opportunistic infection, often a threat to immunocompromised patients.

Assays for IL-12R expression may be conventional immunochemical assays for cell surface antigens. Peripheral blood mononuclear cells can be isolated from patient and incubated with IL-12R monoclonal antibody under conditions which allow the antibody to bind the surface antigen. Antibody bound to the cell
20   surface provides a measure of IL-12R expression. Binding of the antibody to cells may be evaluated by employing an IL-12R monoclonal antibody labelled with a radioactive, fluorescent or other compound capable of being detected.

The invention also involves a method for detecting soluble IL-12 receptor concentration in samples derived from subjects with immune system disorders, cancer, or other diseases that would be marked by
25   an increase or decrease in soluble form of IL-12R. Assays for soluble IL-12R may be conventional sandwich immunochemical assays or ^{125}I-IL-12 binding assays to immobilized IL-12R.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are
30   possible within the scope of the invention. Certain embodiments of this invention are exemplified in the Examples which follow. The Examples are set forth to aid in an understanding of the invention but are not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow.

35   MATERIALS AND METHODS

Proteins and Plasmids

Recombinant human IL-12 (U. Gubler et al., Proc. Natl. Acad. Sci. USA (1991) 88, 4143, and murine IL-
40   12 (D. Schoenhaut et al., J. Immunology (1992), 148, 3433) were obtained as described therein.

The murine anti human IL-12 receptor monoclonal antibody 2-4E6 used herein was generated as described herein below and was purified from ascites fluids by affinity chromatography on protein G-agarose according to the manufacturer's instructions (Genex). The proteins were labelled with I-125 by a modification of the Iodogen method as described (Pierce Chemical Co., Rockford, IL). Radiospecific
45   activities of 5000 - 7000 cpm/fmole for IL-12 and 1500 - 2500 cpm/fmole for the 2-4E6 antibody were typically obtained.

The plasmid pEF-BOS is based on a pUC 119 backbone and contains the elongation factor 1 alpha promoter to drive expression of genes inserted at the BstXI site (S. Mizushima and S. Nagata (1990) Nucl. Acids Res. 18, 5322).

50
Example 1

Preparation. Charact rization and Purification of Hybridoma Antibodies

55   Balb/c mice (Charl s Riv r Laboratori s) w r immunized by th intraperitoneal rout with PHA-(phytoh magglutanin)-activated human PBMC (PHA-activated PBMC) at 6 x 10^7 cells/mouse. Mic received 5 subs qu nt boost r injections of betwe n 2-5 x 10^7 cells ov r a six month period. For pr paration of activated spl n cells, 2 mic w r inj cted intraperitoneally and intrav nously with 1 x 10^7 and 2.5 x 10^6

cells, r spectively, on two succ ssive days, starting four days prior to the c ll fusion. Spleen cells wer isolated from these mice and fused with SP2/0 (ATCC CRL 1581) c lls at a ratio of 1:1 with 35% v/v poly thyl n glycol 4000 (E. M rck) acc rding to the m thod of Faz kas t al., J. Immunol. Methods (1980) 35, 1. Th fus d cells wer plat d at a density of 6 x 10⁵ cells/ml/w ll in 48-w ll clust r dish s in IMDM

5 supplemented with 10% FBS, glutamine (2 mM), β-mercaptoethanol (0.1 mM), gentamicin (50 g/ml), 5% ORIGEN hybridoma cloning factor (IGEN, Inc.), 5% P388D1 (ATCC CRL 46) supernatant (Nordan, R.P., et al., J. Immunol. (1987) 139, 813) and 100 Units/ml rHuIL-6. Hybridoma supernatants were assayed for specific anti-IL-12 receptor antibodies by: 1) immunoprecipitation of the soluble complex of ¹²⁵I-HuIL-12 crosslinked to IL-12 receptor (125I-IL-12/IL-12R), 2) inhibition of ¹²⁵I-HuIL-12 binding to PHA-activated

10 PBMC's, and 3) differential binding to IL-12 receptor positive cells versus receptor negative cells). Hybridoma cell lines secreting specific anti-receptor antibodies were cloned by limiting dilution. Antibodies were purified from ascites fluids by affinity chromatography on Protein G bound to cross-linked agarose according to the manufacturer's protocol (Genex).

15 Example 2

Preparation of Human PHA Lymphoblasts and IL-12 Receptor Binding Assays

Human peripheral blood mononuclear cells were isolated (see Gately et al, J. Natl. Cancer Inst. 69,
20 (1982) 1245) and cultured at 37°C at a density of 5 x 105 cells/ml in tissue culture medium (TCM) containing 0.1% PHA-P (Difco). After 3 days, the cultures were split 1:1 with fresh TCM, and human rIL-2 was added to each culture to give a final concentration of 50 units/ml. The cultures were then incubated for an additional 1-2 days, prior to use in assays.

PHA-activated human PBMC were washed once in binding buffer (RPMI-1640, 5% FBS, 25 mM HEPES
25 pH 7.4) and resuspended in binding buffer to a cell density of 7 x 10⁶ cells/ml. Lymphoblasts (7 x 10⁵ cells) were incubated with various concentrations of ¹²⁵I-IL-12 (5-10000 pM) at room temperature for the designated times. Cell bound radioactivity was separated from free ¹²⁵I-IL-12 by centrifugation of the assay mixture through 0.1 ml of an oil mixture (1:2 mixture of Thomas Silicone Fluid 6428-R15 : A.H. Thomas, and Silicone Oil AR 200 : Gallard-Schlessinger) at 4°C for 90 sec at 10,000 x g. The tip containing the cell
30 pellet was excised, and cell bound radioactivity was determined in a gamma counter. Non-specific binding was determined by inclusion of 100 nM unlabelled IL-12 in the assay. Incubations were carried out in duplicate or triplicate. Receptor binding data were analyzed by using the non-linear regression programs EBDA and LIGAND as adapted for the IBM personal computer by McPherson, J. Pharmacol. Methods (1985) 14, 213 from Elsevier-BIOSOFT.

35

Example 3

Affinity Cross-Linking of ¹²⁵I-IL-12 to IL-12 Receptor Bearing Cell Lines

40 IL-12 receptor bearing cells were incubated with ¹²⁵I-IL-12 (100-500 pM) in the presence or absence of excess unlabelled IL-12 for 2 hr at room temperature. The cells were then washed with ice-cold PBS pH 8.3 (25 mM Sodium Phosphate pH 8.3, 0.15 M NaCl and 1 mM MgCl₂) and resuspended at a concentration of 0.5-1.0x10⁷ cells/ml in PBS pH 8.3. BS3 (Pierce) in dimethyl sulfoxide was added to a final concentration of 0.4 mM. Incubation was continued for 30 min. at 4°C with constant agitation. The cells were washed with
45 ice-cold 25 mM Tris-HCl (pH 7.5), 0.15 m NaCl and 5 mM EDTA and then solublized at 0.5 - 1.0 x 10⁸ cells/ml in solubilization buffer (50 mM Tris-HCl (pH 8.0) containing 8 mM CHAPS, 0.25 M NaCl, 5 mM EDTA, 40 µg/ml PMSF, 0.05% NaN₃, and 1% BSA) for 1 hr at 4°C. The extracts were centrifuged at 12,000 x g for 45 min. at 4°C to remove nuclei and other debris.

50 Example 4

Immunoprecipitation Assay of the Soluble Complex of ¹²⁵I-IL-12 Crosslinked to Human IL-12R

For th immunoprecipitation assay, hybridoma cultur sup rnatant (0.5 ml), diluted antisera, or purified
55 IgG was add d to a microfuge tub containing 0.1 ml of a 50% suspension of ith r goat-anti-mous IgG coupled to agarose (SIGMA CHEM. CO.) or Prot in G coupled to Sepharos 4B (Pharmacia). The assay volum was brought up to 1.0 ml with IP buff r (8 mM CHAPS in PBS (0.25 M NaCl), 1% BSA, & 5 mM EDTA) and th mixtur was incubated on a rotating mixer for 2 hr at room t mperatur . Th beads w re

pelleted by centrifugation, r susp nded in 1 ml IP buffer containing ^{125}I-IL-12/IL-12R (10-20,000 cpm) and the mixtur was incubated on a rotating mixer for 16 hr at 4°C. After this incubation, the beads wer pell ted by c ntrifugation and washed twic in IP buff r without BSA. Th ^{125}I-lab lled rec ptor complex bound to th solid phas antibodi s was r l ased by adding 100 μl of 2x La mmli sampl buff r (Nature 227, 680 (1970)) with and without 10% β-mercaptoethanoland heating for 5 min. at 95°C. The immunoprecipitated proteins were analyzed by SDS-PAGE on 8% or 4-15% gradient polyacrylamide gels and visualized by autoradiography.

Example 5

Assays for IL-12R Solubilized from Cells Expressing IL-12 Receptor.

To confirm that the antibodies identified by the immunoprecipitation assay were specific for IL-12R, an immunoprecipitation/soluble IL-12R binding assay was developed. As described in Example 1 above, antibodies (as hybridoma supernatant, purified IgG (50 μg) or antisera) were immobilized by binding to goat anti-mouse IgG coupled to agarose (100 μl; Sigma Chemical Co.) or protein G coupled to Sepharose 4B (100 μl; Pharmacia). For some experiments, antibodies were covalently crosslinked to protein G-Sepharose 4B, before being used in the assay. See Stern and Podlaski, Techniques in Protein Chemistry (1993). The immobilized antibodies were resuspended in IP buffer (0.3 ml) and 0.2 ml of a detergent solubilized extract of PHA-activated PBMCs or K6 cells that contained IL-12R was added. To prepare the detergent solubilized IL-12R preparation, the cells were washed with ice-cold 25 mM Tris-HCl (pH 7.5), 0.15 M NaCl and 5 mM EDTA and then solublized at 1.5×10^8 cells/ml in solubilization buffer (50 mM Tris-HCl, pH 8.0, containing 8 mM CHAPS, 0.25 M NaCl, 5 mM EDTA, 40 μg/ml PMSF, 0.05% NaN_3, and 1% BSA) for 1 hr at 4°C. The extracts were centrifuged at 120,000 x g for 60 min. at 4°C to remove nuclei and other debris. The mixture was incubated on a rotating mixer for 16 hr at 4°C. After this incubation, the beads were pelleted by centrifugation and resuspended in IP buffer (0.15 ml) containing ^{125}I-HuIL-12 at concentrations ranging from 0.05 to 7.5 nM. The IL-12R immobilized on the antibody coated beads was incubated with ^{125}I-HuIL-12 for 2 hrs. at room temperature on a shaker. Following this incubation, the beads were pelleted, washed twice with IP buffer and the bound radioactivity determined in a gamma counter. Nonspecific binding was determined by inclusion of 70 nM unlabelled human IL-12 in the assay. Solubilized IL-12R binding data were analyzed according to the method of Scatchard, (Assn. N.Y. Acad. Sci. 51, 660 (1949)) by using the nonlinear regression programs EBDA and Ligand as adapted for the IBM PC by McPherson, supra from Elsevier-BIOSOFT.

Example 6

Competitive Inhibition of ^{125}I-IL-12 Receptor Binding by Antibodies

The ability of hybridoma supernatant solutions, purified IgG, or antisera to inhibit the binding of ^{125}I-IL-12 to PHA-activated lymphoblasts was measured as follows: serial dilutions of culture supernatants, purified IgG or antisera were mixed with activated lymphoblasts ($1-1.5 \times 10^6$ cells) in binding buffer (RPMI-1640, 5% FBS + 25 mM HEPES pH 7.4) and incubated on an orbital shaker for 1 hour at room temperature. ^{125}I-HuIL-12 (1×10^5 cpm) was added to each tube and incubated for 1-2 hours at room temperature. Nonspecific binding was determined by inclusion of 10 nM unlabelled IL-12 in the assay. Incubations were carried out in duplicate or triplicate. Cell bound radioactivity was separated from free ^{125}I-IL-12 by centrifugation of the assay through 0.1 ml of an oil mixture as described above. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter.

Example 7

Labelling of Human IL-12 and Mab 2-4E6 with [125]I

Human IL-12 and purified 2-4E6 (Example 13) IgG wer labelled with ^{125}I by a modification of the Iodog n m thod (Pi rce Chemical Co., Rockford, IL). Iodog n was dissolv d in chloroform and 0.05 mg dri d in a 12 x 15 mm borosilicate glass tub . For radiolabeling, 1.0 mCi Na[^{125}I] (Am rsham, Chicago, IL) was added to an Iodogen-coated tube containing 0.05 ml of Tris-iodination buff r (25 mM Tris-HCl pH 7.5, 0.4 M NaCl and 1 mM EDTA) and incubated for 4 min at room temperatur . Th activated ^{125}I solution was transferred to a tube containing 0.05 to 0.1 ml IL-12 (7 μg) or IgG (100 μg) in Tris-iodination buffer and th

reaction was incubat d for 9 min. at room t mp rature. At the nd of th incubation, 0.05 ml of Iodogen stop buff r (10 mg/ml tyrosine 10% glycerol in Dulbecco's PBS, pH 7.40) was added and reacted for 3 min. Th mixtur was then dilut d with 1.0 ml Tris-iodination buffer, and applied to a Bio-G l P10DG d salting column (BioRad Laboratories) for chr matography. The column was eluted with Tris-iodination buff r, and
5 fractions (1 ml) containing the peak amounts of labelled protein were combined and diluted to 1 x 108 cpm/ml with 1% BSA in Tris-iodination buffer. The TCA preciptable radioactivity (10% TCA final concentration) was typically in excess of 95% of the total radioactivity. The radiospecific activity was typically ~ 1500 to 2500 cpm/fmol for 2-4E6 IgG and 5000 to 7000 cpm/fmol for IL-12.

10 Example 8

Binding Assays of ^{125}I-2-4E6 to Intact Cells

PHA-activated human PBMC were washed once in binding buffer (RPMI 1640, 5% FBS and 25 mM
15 HEPES, pH 7.4) and resuspended in binding buffer to a cell density of 1.5×10^7 cells/ml. Lymphoblasts (1.5 $\times 10^6$ cells) were incubated with various concentrations of ^{125}I-2-4E6-IgG (0.005 to 2 nM) at room temperature for 1.5 hrs. Cell bound radioactivity was separated from free ^{125}I-2-4E6 IgG by centrifugation of the assay mixture through 0.1 ml silicone oil at $4 \degree C$ for 90 seconds at 10,000 x g. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter. Non-specific
20 binding was determined by inclusion of 67 nM unlabelled 2-4E6 IgG in the assay. Incubations were carried out in duplicate or triplicate. Receptor binding data were analyzed by using the nonlinear regression programs EBDA, Ligand and Kinetics as adapted for the IBM personal computer by McPherson, supra from Elsevier BIOSOFT.

25 Example 9

Expression of Recombinant IL-12R in COS Cells and Determination of ^{125}I-2-4E6 Binding

COS cells ($4-5 \times 10^7$) were transfected by electroporation with 25 μg of plasmid DNA expressing
30 recombinant human IL-12R, as describe herein below, in a BioRad Gene Pulser (250 μF, 250 volts) according to the manufacturer's protocol. The cells were plated in a 600 cm² culture plate, harvested after 72 hours by scraping, washed and resuspended in binding buffer. Transfected cells (8×10^4 were incubated with increasing concentrations of ^{125}I-labelled 2-4E6 or IL-12 at room temperature for 2 hrs. Cell bound radioactivity was separated from free ^{125}I-labelled 2-4E6 or IL-12 as described above.

35
Example 10

Western Blot Analysis of Soluble IL-12R with mAb 2-4E6

40 PHA-activated PBMC were washed 3 times with ice-cold PBS and solubilized at $0.5 - 1 \times 10^8$ cells/ml in solubilization buffer (50 mM Tris-HCl pH 8.0 containing 8 mM CHAPS, 0.25 M NaCl, 5 mM EDTA, 40 μg/ml PMSF, 0.05% NaN₃ and 1 mg/ml BSA) for 1 hr at $4 \degree C$. The extracts were centrifuged at 12,000 x g for 45 min. at $4 \degree C$ to remove nuclei and other debris. The extracts were incubated with 2-4E6 IgG or control IgG bound to goat-anti-mouse IgG immobilized on cross-linked agarose (Sigma Chemical Co.). The precipitated
45 proteins were released by treatment with 0.1 M glycine pH 2.3, neutralized with 3M Tris, mixed with 1/5 volume of 5 x Laemmli sample buffer, and separated by SDS/PAGE on 8% pre-cast acrylamide gels (NOVEX). The separated proteins were transferred to nitrocellulose membrane (0.2 μM) for 16 hours at 100 volts in 10 mM TRIS-HCl (pH 8.3), 76.8 mM glycine, 20% methanol and 0.01% SDS. The nitrocellulose membrane was blocked with BLOTTO (5.0% w/v nonfat dry milk in PBS + 0.05% Tween 20) and duplicate
50 blots were probed with ^{125}I-2-4E6 IgG (1 x 106 cpm/ml in 8 mM CHAPS in PBS, 0.25 M NaCl, 10% BSA and 5 mM EDTA) + unlabelled 2-4E6 IgG (67 nM).

55

Exampl 11

Analysis of IL-12 Rec ptor Expr ssion on Human C lls by Fluorescenc Activated C ll Sorting with mAb 2-4E6

5

    To stain cells expressing IL-12 receptor, 1 x 10⁶ cells in 100 μl staining buffer (PBS containing 2% FBS and 0.1% NaN₃) were incubated with 10 μl of 2-4E6 ascites fluid for 25 min. at 4°C. Cells were then washed twice with staining buffer followed by incubation with a 1:100 dilution of goat F(ab)2 anti mouse Ig-PE (Tago, Burlingame CA) for 25 min. at 4°C. The stained cells were washed twice with staining buffer and
10 then analyzed on a FACScan flow cytometer (Becton Dickinson).

Example 12

Inhibition of IL-12 Binding to Human PHA-Lymphoblasts by Mouse Anti-IL-12R Antiserum

15

    Mice immunized with PHA-activated PBMCs developed an immune response against the human IL-12R as determined by inhibition of ^{125}I-IL-12 binding to PHA-activated PBMCs (Figure 7) and immunoprecipita-tion of the complex of ^{125}I-IL-12 crosslinked to IL-12R (data not shown). The dilutions for half-maximal inhibition of ^{125}I-IL-12 binding to PHA-activated PBMCs were 1/500 and 1/250 for animals 211-1 and 211-2,
20 respectively (Figure 7). These antisera also neutralized IL-12 biologic activity as measured in a PHA-lymphoblast proliferation assay (data not shown). Spleen cells isolated from these mice were fused with SP2/0 myeloma cells and the resulting hybridomas were initially screened for IL-12R specific antibodies by immunoprecipitation of the ^{125}I-IL-12/IL-12R complex and by inhibition of ^{125}I-IL-12 binding to IL-12R.
    For Figure 7, ten fold serial dilutions of mouse anti-IL-12R immune serum (#211-1 and #211-2) and
25 normal mouse serum (NMS) were preincubated with PHA-activated PBMC for 60 min at RT (room temperature) before addition of ^{125}I-IL-12 (100 pM). After addition of ^{125}I-IL-12, the reaction was incubated for 1-2 hrs at RT and the cell bound radioactivity was determined. The data are expressed as the % Inhibition of ^{125}I-IL-12 binding in the presence of the immune serum when compared to the specific binding in the absence of serum.

30

Example 13

Identification and Characterization of Monoclonal Anti-IL-12R Antibodies

35   The immunoprecipitation assay identified 13 hybridomas secreting putative non-neutralizing anti-IL-12R antibodies, whereas the IL-12R binding assay identified 3 putative neutralizing IL-12R antibodies (Table 1). The immunoprecipitation assay measured the ability of the putative anti-IL-12R antibodies that are immobilized on a solid phase to capture the solubilized complex of ^{125}I-IL-12/IL-12R. To verify that the radioactivity immunoprecipitated by the immobilized antibody was present in the complex of ^{125}I-IL-12/IL-
40 12R, the immunoprecipitated proteins were solubilized, separated by SDS-PAGE and visualized by autoradiography. The preparations of the ^{125}I-IL-12/IL-12R complexes solubilized from PHA-activated PBMC, Kit-225 and K6 cells were resolved into two major radioactive bands, 210-250 kDa and 75 kDa (Figure 8). The 210-250 kDa and 75 kDa complexes were identified as the ^{125}I-IL-12/IL-12R complex and ^{125}I-IL-I2 not complexed with the receptor, respectively (Figure 8). See also Chizzonite et al., J. Immunol. 148, 3117·
45 (1992). The radioactive 75 kDa band visualized from the cell extracts co-migrated with ^{125}I-IL-12, indicating that it represented ^{125}I-IL-12 that bound but was not covalently crosslinked to IL-12R. The 210-250 kDa band was not a covalent crosslinked oligomer of ^{125}I-IL-12 because it is not produced when the crosslinking agent BS3 was added directly to ^{125}I-IL-12 (Figure 8).
    Hybridoma cells secreting putative anti-IL-12R antibodies were then cloned by limiting dilution and
50 screened by both the immunoprecipitation and inhibition of binding assays that identify non-neutralizing and neutralizing antibodies, respectively. During this cloning and screening process, hybridoma lines secreting putative neutralizing anti-IL-12R antibodies were not recovered, whereas non-neutralizing antibodies were recov red from both th original immunoprecipitation and inhibitory positive hybridomas. Aft r this initial id ntification and cloning, a dir ct binding assay was used to det rmine if th non-n utralizing antibodies
55 only bound to cells expr ssing IL-12R. This assay d monstrat d that th non-n utralizing antibodies could be divided into 2 classes, those that bound only IL-12R positive human c lls and those that bound to most human c lls (data not shown). R pres ntativ antibodi s from ach class, 2-4E6 and 2C6, r spectiv ly, wer produced in ascites fluid, purified by protein G affinity chromatography and xt nsiv ly characteriz d.

For Figure 8, PHA-activat d PBMC (PHA-PBMC), Kit-225 (Kit-225) and K6 (K6) cells (1 x 10^7 c lls/ml) were incubated with ^{125}I-IL-12 (100-500 pM) for 2 hrs at room temp rature in the absence or pr s nc of 25 nM unlab lled IL-12. Cells w r th n wash d, affinity crosslinked with BS3 (0.4 mM final conc ntration) and a cell xtract pr pared as d scrib d. Th c ll xtract was precipitated with wh at germ l ctin bound to solid
[5] supports as described. The precipitated proteins were released by treatment with sample buffer and analyzed by SDS-PAGE and autoradiography on a 8.0% slab gel. The complex of ^{125}I-IL-12 crosslinked to the IL-12 receptor migrates as a single major band of approximately 210-250 kDa. The band migrating at 75 kDa is ^{125}I-IL-12 that was bound but not crosslinked to the IL-12 receptor. ^{125}I-IL-12 (IL-12) and ^{125}I-IL-12 that was treated with the BS3 crosslinker (IL-12/BS3) were electrophoresed in parallel lanes as markers for
[10] the migration of the 75 kDa IL-12 heterodimer and for any oligomers of IL-12 that may form with the BS3 crosslinker. The molecular sizes indicated in the margins were estimated from standards run in parallel lanes. Exposure time was 7 days.

Table 1

INITIAL IDENTIFICATION OF HYBRIDOMAS SECRETING ANTI-IL-12 RECEPTOR ANTIBODIES: SPLENOCYTES FROM MICE #211-1 AND #211-2			
HYBRIDOMA/ANTIBODY I.P. ASSAY[1] (cpm bound)			INHIBITION ASSAY[2]
IL-12R 2C6[3]		1900	-
211-1	1A5	722	-
	4E6	840	-
	5C1	312	+
211-2	3B1	1323	-
	4A3	2172	-
	4D6	804	-
	5D5	877	-
	4A5	509	+
	4C6	456	+
	1D1	1395	-
	5E6	2043	-
	2-4E6	2836	-
Control mAb 402		-	

[1] I.P. assay measures the amount of ^{125}I-IL-12/IL-12R complex bound by the immobilized antibody.
[2] Inhibition assay measures whether the antibody can inhibit ^{125}I-IL-12 binding to PHA-activated PBMC.
[3] IL-12R 2C6 is an antibody that both immunoprecipitates the ^{125}I-IL-12/IL-12R complex and binds to many IL-12R positive and negative human cells. This antibody probably recognizes a component closely associated with the IL-12R.

[45] Example 14

Characteristics of Monoclonal Anti-IL-12R Antibody 2-4E6 Binding to Natural IL-12R

mAb 2-4E6 immunoprecipitates the ^{125}I-IL-12/IL-12R complex solubilized from PHA-activated human
[50] lymphoblasts, Kit-225 and K6 cells (Figure 9, data shown for PHA-activated PBMC), but does not block ^{125}I-IL-12 binding to IL-12R expressed on these cells. These data suggested that the 2-4E6 antibody was a non-inhibitory or non-neutralizing anti-IL-12R antibody. To confirm that 2-4E6 was an non-inhibitory antibody specific for th IL-12R, 2-4E6 was labelled with ^{125}I and direct binding assays were performed with IL-12R positiv and negativ cells. ^{125}I-2-4E6 binds to IL-12R bearing c lls with an affinity that rang s from 337 pM
[55] to 904 pM and identifies betwe n 1500 and 5000 binding sites per cell (PHA-activated PBMC, Figure 10; K6 cells, Figure 11). IL-12 does not block ^{125}I-2-4E6 from binding to PHA-activated PBMCs and confirms that 2-4E6 is a non-inhibitory/non-n utralizing antibody (Figur 12). ^{125}I-2-4E6 binds to other c lls xpr ssing IL-12R, such as Kit 225, and YT c lls, but does not bind to IL-12R negativ c lls (non-activated human PBMC,

MRC-5 fibroblasts and HL-60 cells (Tabl 2).

Equilibrium binding assays have demonstrated that ^{125}I-IL-12 id ntifies 3 separate binding sites on the surfac of PHA-activat d PBMCs, Kit-225 and K6 cells (Figure 13, data for K6 cells and Tabl 2). Analysis of this binding data by the method of Scatchard, supra shows th s affinities are approximately 5-20 pM, 50-200 pM and 2-6 nM, respectively. The total number of ^{125}I-IL-12 binding sites per cell are approximately 1500 to 5000, which is in good agreement with the total number of binding sites identified by ^{125}I-2-4E6 (Table 2). The data also suggests that 2-4E6 recognizes the low affinity (2-5 nM) binding component of the IL-12 receptor in much the same manner that the anti-TAC antibody recognizes the low affinity component (p55 subunit) of the IL-2 receptor.

Since the data indicated that mAb 2-4E6 was a non-neutralizing antibody specific for the IL-12R, the molecular weight and ^{125}I-IL-12 binding characteristics of the protein(s) immunoprecipitated by mAb 2-4E6 from the surface of IL-12R positive cells was investigated. The steady state binding of ^{125}I-IL-12 to proteins immunoprecipitated by immobilized 2-4E6 from solubilized extracts of PHA-activated PBMCs, Kit-225 and K6 cells was saturable and specific (Figure 14, data for extracts from K6 cells). Transformation of the binding data by the method of Scatchard, revealed a single site with an apparent affinity of 188 pM. The proteins immunoprecipitated by 2-4E6 from the cell extracts were resolved by SDS-PAGE, transferred to nitrocellulose membrane and probed with ^{125}I-2-4E6 in a western blot. On the western blot, ^{125}I-2-4E6 binds to an approximately 90 kDa protein, that is only immunoprecipitated by 2-4E6 and not by an anti-IL-12 antibody or a control antibody (Figure 15, data shown for PHA-activated PBMCs). In summary, all the data demonstrated that mAb 2-4E6 bound a protein on the surface of IL-12R positive cells that was approximately 90 kDa and bound ^{125}I-IL-12 in a specific manner.

For Figure 9, soluble complexes of ^{125}I-IL-12/IL-12R were prepared from PHA-activiated human PBMC as detailed herein (see also Figure 8) and immunoprecipitated by immobilized antibodies, 2-4E6, 2C6, 4D6, 20C2 and control. The soluble complexes were also precipitated with wheat germ lectin immobilized on crosslinked agarose. The precipitated proteins were analyzed as described herein and in Figure 8. Antibodies 4D6 and 20C2 are non-neutralizing and neutralizing anti-IL-12 antibodies, respectively. 4D6 immunoprecipitates ^{125}I-IL-12/IL-12R complex and free ^{125}I-IL-12, whereas 20C2 only immunoprecipitates free ^{125}I-IL-12. Both 2-4E6 and 2C6 recognize the ^{125}I-IL-12/IL-12R complex. ^{125}I-IL-12 (IL-12) and ^{125}I-IL-12 that was treated with the BS3 crosslinked (IL-12/BS3) were electrophoresed in parallel lanes as markers for the migration of the 75 kDa IL-12 heterodimer and for any oligomers of IL-12 that may form with the BS3 crosslinker. The molecular sizes indicated in the margins were estimated from standards run in parallel lanes. Exposure time was 7 days.

For Figure 10, Lymphoblasts (1 x 10^6 cells) were incubated for 2 hrs at room temperature with increasing concentrations of ^{125}I-2-4E6 in the absence (°) or presence (•) of 25 nM unlabelled 2-4E6. Total (°) and non-specific (•) cell bound radioactivity were determined as described. Specific binding of ^{125}I-2-4E6 (▽) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard as determined by Ligand computer program with a single-site model.

For Figure 11, K6 cells (1 x 10^6 cells) were incubated for 2 hrs at room temperature with increasing concentrations of ^{125}I-2-4E6 in the absence (•) or presence (▽) of 25 nM unlabelled 2-4E6. Total (•) and non-specific (▽) cell bound radioactivity were determined as described. Specific binding of ^{125}I-2-4E6 (▼) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard as determined by Ligand with a single-site model.

For Figure 12, The data are expressed as the amount of ^{125}I-2-4E6 bound [CPM BOUND (Percent)] to the cells in the presence of the indicated concentrations of unlabelled antibody or IL-12 when compared with the total specific binding in the absence of unlabelled competitor.

For Figure 13, K6 cells (1 x 10^6 cells) were incubated for 2 hrs at room temperature with increasing concentrations of ^{125}I-IL-12 in the absence (°) or presence (•) of 50 nM unlabelled IL-12. Total (°) and non-specific (•) cell bound radioactivity were determined as described. Specific binding of ^{125}I-IL-12 (▽) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard as determined by Ligand with a single-site model.

For Figure 14, K6 cells 1.5 x 10^8 cells/ml) were solubilized with 8 mM CHAPS extraction buffer and the c ll xtract (0.2 ml) was immunoprecipitated for 16 hrs at 4°C with mAb 2-4E6 immobilized on goat anti-mouse IgG coupled to agaros as d scribed. Following this incubation, th beads w r pelleted, washed and r suspended in IP buffer containing ^{125}I-IL-12 at concentrations ranging from 7 pM to 7.5 nM. Th IL-12R immobilized on the 2-4E6 coated beads was incubated with ^{125}I-IL-12 for 2 hrs at RT and IL-12R bound radioactivity was d t rmin d in th pr senc of 50 nM unlabell d IL-12. Th right hand pan ls show analysis of th binding data according to the method of Scatchard as det rmined by Ligand with a single-

site mod I.

For Figure 15, PHA-activated PBMC (1 x 10^8 cells/ml) wer solubilized with 8 mM CHAPS extraction buff r and th c ll xtract (1 ml) was immunopr cipitated as d scribed in Figur 13. Following this incubation, th beads w r pelleted, wash d and th bound prot ins r l as d by treatm nt with 0.1 M glycine pH 2.3. The released proteins were separated by non-reducing SDS/PAGE on 8% gels transferred to nitrocellulose membrane and probed with ^{125}I-2-4E6 as described. The molecular sizes indicated in the margins were estimated from molecular weight standards (Amersham Prestained High Molecular Weight Standards) run in parallel lanes. Exposure time was 7 days.

Table 2

COMPARISON OF THE BINDING OF IL-12 AND 2-4C6 TO HUMAN CELLS EXPRESSING IL-12 RECEPTOR				
CELL TYPE	IL-12 BINDING[1]		2-4E6 BINDING[2]	
Human Cells	K_D (nM)	Sites/cell	K_D (nM)	Sites/cell
non-activated human PBMC[3]	none	detected	none	detected
PHA-PBMC (5-7 days)(3 sites)	0.018 0.084 1.800	312 501 1406	0.745	1472-2246
K6 cells (3 sites)	0.016 0.057 2.400	707 939 4036	0.489	3116-5259
Kit-225 (3 sites)	0.023 0.210 2.360	100 250 755	0.594	1950
YT cells (2 sites)	0.006 0.109	24 117	0.904	4522
RAJI cells MRC-5 HL-60	none none none	detectable detectable detectable	0.450 none none	561 detectable detectable

[1] Steady state ^{125}I-IL-12 binding assays. Apparent dissociation constant (K_D) and binding sites per cell have been calculated by transformation of the data by the method of Scatchard.

[2] Steady state ^{125}I-2-4E6 binding assays. Data transformed by the method of Scatchard.

[3] Human peripheral blood mononuclear cells (PBMC) were activated with PHA as described in the methods (PHA-PBMC).

Example 15

mAb 2-4E6 Binding To Human Recombinant IL-12R Expressed in COS Cells

The characteristics of the protein bound by mAb 2-4E6 fulfilled standard criterion for an IL-12R and therefore 2-4E6 was used in an expression cloning strategy to isolate a cDNA coding for the human IL-12R. A cDNA coding for the human IL-12R was isolated by this method. The IL-12R cDNA was engineered in a mammalian cell expression vector, transfected into COS-7 (ATCC CRL 1651) cells and the specificity for binding of ^{125}I-IL-12 and ^{125}I-2-4E6 was determined. Steady state binding of ^{125}I-IL-12 to the rIL-12R expressing COS cells identifies a single binding site with an apparent affinity of 2-6 nM and approximately 150,000 sit s/cell (Figur 4A). This low affinity IL-12 binding sit corr sponds to th low affinity sit seen in th binding assays with human c lls that naturally xpr ss IL-12R. The binding of 125I-2-4E6 to rIL-12R expressed in th COS c lls is saturabl and specific and id ntifi s approximately 500,000 sites/c ll (Figure 4B). COS c lls transf cted with an unr lated plasmid do not bind ith r ^{125}I-IL-12 or ^{125}I-2-4E6 (data not shown). These data demonstrated unequivocally that mAb 2-4E6 was specific for the low affinity compon nt of th IL-12R.

For Figur 4A, COS c lls w re transf cted with a plasmid expressing human rIL-12R as d scrib d. Three days later, transfected cells (1 x 10⁴ c lls) w re incubated for 2 hrs at room temp rature with incr asing concentration of ¹²⁵I-IL-12 in th abs nc (O) or pr s nc (□) of 50 nM unlabelled IL-12. Total (O) and non-specific (□) cell bound radioactivity w r determin d as d scribed. Specific binding of ¹²⁵I-

5 IL-12 (▲) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard as determined by Ligand with a single-site model.

For Figure 4B, COS cells were transfected with a plasmid expressing human rIL-12R as described. Three days later, transfected cells (1 x 10⁴ cells) were incubated for 2 hrs at room temperature with

10 increasing concentrations of ¹²⁵I-2-4E6 in the absence (O) or presence (□) of 50 nM unlabelled 2-4E6. Total (O) and non-specific (□) cell bound radioactivity were determined as described. Specific binding of ¹²⁵I-2-4E6 (▲) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard as determined by Ligand with a single-site model.

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Example 16

Analysis of mAb 2-4E6 Binding to IL-12R Positive Human Cells by Fluorescence Activated Cell Sorting (FACS)

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The expression level of IL-12R on human cells could be regulated depending on the activation state of the cells, the cell cycle or the type of environment from which the cells are isolated. Previous data had demonstrated that PHA activation of PBMC leads to a gradual rise in IL-12R expression, reaching a maximum at 3-4 days after activation and declining thereafter. Desai et al. (1992) J. Immunol., 148, 3125.

25 To investigate the heterogeneity of IL-12R expression on PHA-activated PBMCs, Kit-225 and K6 cells, · FACS analysis of IL-12R on these cells was determined with mAb 2-4E6 (Figure 16). The fluorescence intensity of binding of 2-4E6 was specific and indicated that these three cell types expressed approximately equal numbers of IL-12R. Interestingly, the FACS analysis indicated that the cell population was fairly homogenous and did not have one population expressing no or low numbers of IL-12R and a second

30 population that expressed very high numbers of IL-12R.

For Figure 16, Day 4 PHA-activated lymphoblasts, Kit-225 and K6 cells were analyzed for IL-12R expressing cells by the indirect fluorescent antibody-labelling technique described. The figure depicts specific staining for IL-12R obtained in the presence of mAb 2-4E6 (IL-12R) and non-specific staining obtained in the presence of a control antibody specific for IL-1 receptor (anti-HuIL-1R), a control antibody

35 specific for human IL-12 (4D6 + GART-PE CTRL) and the goat anti-mouse antibody conjugated with PE (GART-PE CTRL).

Example 17

40 ## Cell Culture

Peripheral blood mononuclear cells (PBMC) were isolated from blood collected from healthy donors. The blood was collected into heparinized syringes, diluted with an equal volume of Hank's balanced salt solution (HBSS) and layered over Ficoll-Hypaque. The tubes were spun at 2000 rpm for 20 minutes at room

45 temperature. PBMC at the interface were collected and pelleted at 1500 rpm for 10 minutes through a 15 ml cushion of 20% sucrose in PBS. Pelleted PBMC were resuspended in tissue culture medium and washed twice in the same medium (RPMI 1640 plus 5 % serum). Finally, the cells were cultured at 0.5 - 1 x 10⁶ cells/ml in tissue culture medium plus 1 µg/ml PHA-P (Difco) for 3 days at 37°C in a 5% CO₂ atmosphere. Cells were split 1:1 in culture medium plus 50 U/ml rhuIL-2 (Roche) to yield >95% T-cells. The next day,

50 these cells were used for assessing their responsiveness to IL-12, for radioligand (IL-12) binding assays and in flow cytometry assays for the detection of IL-12 receptors.

Flow cytometric detection of IL-12 receptors on such 4 day activated PHA blasts was performed as follows: the c lls wer wash d twice in PBS and r suspended at 2 x 10⁶ cells/ml in PBS plus 2% f tal calf s rum and 0.1% sodium azid . All the subs qu nt st ps w r carri d out at 4°C. 1 x 10⁶ c lls w r

55 incubated in 1 nM human IL-12 for 40 minut s. The cells wer washed in FACS buffer and incubated with about 1 µg of biotinylated rat anti human p40 IL-12 subunit antibody 4D6 (Example 13) for 20 minut s. Cells w r wash d again and r susp nded in 100 µl of a 5 µg/ml str ptavidin-phycoerythritin conjugate (Fish r Biot ch) for 15 minut s. Th c lls were then washed again befor analysis on a FACSAN flow

cytomet r (B cton Dickinson).

Example 18

5   Extraction and characterization of RNA

PHA activated cells as described above were harvested at day 2-3 and total RNA was extracted using GuanidinIsothiocyanate/Phenol as described (P. Chomczynski and N. Sacchi (1987) Anal. Biochem., 162, 156). Poly A+ RNA was isolated from the total RNA by one batch adsorption to oligo dT latex beads as
10   described (K. Kuribayashi et al (1988) Nucl. Acids Res. Symposium Series 19, 61). The mass yield of this purification was about 4%.

RNA blots were performed as follows. RNA was fractionated in 1.2 % agarose gels under denaturing conditions in the presence of 2.2M formaldehyde and subsequently transferred to nitrocellulose as described (Molecular Cloning, a Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis,
15   Cold Spring Harbour Laboratory Press 1989 (hereinafter "Molecular Cloning Manual)). The RNA blots were hybridized (7 x 10^5 cpm/ml, 30 ml) with labelled probe in 5 x SSC (1X SSC = 0.15 M NaCl-0.015 M NaCitrate) - 50 % formamide - 5 x Denhardts solution (1 x Denhardts = 0.02% polyvinylpyrrolidone, 0.02 % Ficoll 400, 0.02% bovine serum albumin fraction V) - 0.3 % SDS - 250 µg/ml denatured salmon sperm carrier DNA at 37°C overnight. The probe was generated by random-primer labelling gel-isolated insert
20   from IL-12 receptor cDNA clone No. 5 as described in the above manual. The blots were first quickly rinsed at room temperature in 2 x SSC , then washed in 0.1 x SSC at 50°C for 30 minutes, dried and exposed to Kodak XAR film at -70°C for 3 days.

Example 19

25

cDNA Library

From the above polyA+ RNA, a cDNA library was established in the mammalian expression vector pEF-BOS as follows: 3 µg of polyA+ RNA were reverse transcribed into single stranded cDNA using
30   RNaseH minus reverse transcriptase (GIBCO BRL Life Technologies Inc., P.O.Box 9418, Gaithersburg, MD 20898). The resulting mRNA-cDNA hybrids were converted into blunt ended double stranded cDNAs by established procedures (U.Gubler and A.Chua, in: Essential Molecular Biology Volume II, T.A. Brown, editor, pp. 39-56, IRL Press 1991). BstXI linkers (A. Aruffo and B. Seed, Proc. Natl. Acad. Sci. USA (1987) 84, 8573) were ligated to the resulting cDNAs and cDNA molecules > 800 base pairs (bp) were selected over a
35   Sephacryl SF500 column. A Sephacryl SF 500 column (0.8 x 29 cm) was packed by gravity in 10 mM Tris-HCl pH 7.8 - 1 mM EDTA - 100 mM NaAcetate. BstXI linkered cDNA was applied to the column and 0.5 ml fractions were collected. A small aliquot of each fraction was run on a 1% agarose gel, the gel was dried down and the size distribution of the radioactive cDNA visualized by exposure of the gel to X-ray film. cDNA molecules larger than 800 bp were selected in this fashion, pooled, concentrated by ethanol
40   precipitation and subsequently ligated to the cloning vector. The cloning vector was the plasmid pEF BOS (see reference supra) that had been cut with BstXI and purified over two consecutive gels. 300 ng of plasmid DNA were ligated to 30 ng of size selected cDNA from above in 60 µl of ligation buffer (50 mM Tris-HCl pH 7.8 - 10 mM MgCl₂ - 10 mM DTT - 1 mM rATP - 25 µg/ml bovine serum albumin) at 15°C overnight. The following day, the ligation reaction was extracted with phenol, 6 µg of mussel glycogen were
45   added, and the nucleic acids were precipitated by ethanol. The precipitate was dissolved in water and the precipitation was repeated, followed by a wash with 80% ethanol. Finally, the pellet was dissolved in 6 µl of water and 1 µl aliquots were subsequently electroporated into E. coli strain DH-10B (BRL). By elec-troporating 5 parallel aliquots in this fashion, a library of about 10 million recombinants was generated for future use.

50

Example 20

Screening for IL-12 Receptor cDNAs by Panning

55   Th basic principle of th panning method has been d scribed in A. Aruffo and B. Seed, Proc. Natl. Acad. Sci. USA (1987) 84, 8573 as discussed below. Ten library aliquots each r pr senting about 50,000 clon s wer plated on LB amp plat s and w re grown up ov rnight. Th n xt day, th colonies from each pool were scraped off into a s parat 50 ml aliquot of LB + amp and the cultures wer grown for another

two hours before plasmid DNA was xtracted using QIAGEN plasmid kits. Th t n s parat DNA pools wer then transfected into COS cells, using th DEAE d xtran technique (2 million COS cells/9 cm diam ter plat and 2.5 μg DNA) (Molecular Cloning Manual). 2 to 3 days lat r, the COS cells were detached from th plat s using 0.5 mM EDTA/0.02% Na Azid in PBS and a singl cell susp nsion was prepar d for ach
5   pool. The monoclonal anti IL-12 receptor antibody 2-4E6 as discussed above was subsequently bound to the cells in suspension (10 μg/ml in PBS-0.5mM EDTA-0.02% Na Azide-5% FCS, 1 hour, on ice). The cell suspension was then spun through a layer of 2% Ficoll in the above buffer (tabletop centrifuge, 1000 rpm, 4 minutes) to eliminate the excess unbound antibody and the cells were gently resuspended in the same buffer. The cells from one pool were subsequently added to one bacterial petri dish (9 cm diameter) that
10 had been coated with polyclonal goat anti mouse IgG (20 μg/ml in 50 mM Tris-HCl pH 9.5, RT/OVERNIGHT (ON)) and blocked with 1% BSA in PBS (37°C/1 hour). COS cells were panned in this way for 2 hours at RT. Nonadhering cells were then gently washed off with PBS and the remaining adherent cells in the dishes lysed by the addition of 0.8 ml of Hirt lysis solution (0.6% SDS-10 mM EDTA). After transferring to Eppendorf tubes, the lysates were made 1 M NaCl, incubated ON at +4°C and then spun at 15,000 rpm
15 for 10 minutes in the cold. The supernatants were extracted with phenol once, 12 μg of mussel glycogen was added and the DNA precipitated twice by adding 0.5 volumes of 7.5 M NH_4OAc and 2.5 volumes of ethanol. The resulting DNA pellet was washed once with 80% ethanol, dried and taken up in 1 μl of distilled H_2O. The entire prep was then electroporated into E. coli strain DH-10B and the resulting colonies grown up ON. This represents one panning cycle. The ten library aliquots were panned each one separately for a
20 total of three cycles.

From the last cycle of each pool, DNA was again extracted and this time transfected into COS cells plated on plastic one-chamber microscopic slides (2 slides per pool). 2-3 days after transfection, to one of the slides was bound labelled human IL-12 (10^6 cpm/ml = 300 pM in RPMI 1640 plus 5% FCS for 2-3 hours at 4°C) and to the other slide labelled monoclonal Ab 2-4E6 (2 x 10^6 cpm/ml = 1 nM in RPMI 1640
25 plus 5% FCS for 1 hour at RT). The slides were washed in PBS, fixed for 40 seconds in a cold mixture of methanol:acetone (7:3) and air dried. The slides were subsequently dipped in Kodak photographic emulsion NTB2, air dried and exposed in a light-tight container for 2-4 days at 4° C. They were developed in Kodak D10 developer according to the manufacturer's instructions and evaluated under a light microscope using a 10 to 40 fold bright field magnification. One of the ten pools, number 5, showed a large number of positive
30 cells both for IL-12 and 2-4E6 binding. E. coli clones from this 3 x panned pool were subsequently picked into a microtiterplate (3 clones per well for a total of 288 clones). Pools representing the 8 rows and 12 columns from this plate were grown up and their plasmid DNA extracted. These 20 preps were transfected separately into COS cells on 12 well plates (10^5 cells well, 4 wells per pool). 2-3 days after the transfection, labelled IL-12 was bound to the cells in two wells (total binding), whereas the other two wells per pool
35 received labelled IL-12 and a 100 fold molar excess of cold IL-12 (= nonspecific binding). Wells were washed and the bound radioactivity eluted with 0.5 ml of 1% SDS and counted in a gamma counter. Two positive pools were identified in this manner, one representing column 1 and the other one representing row F from the microtiterplate. E. coli clones from well F1 must thus contain the IL-12 binding activity. Clones from that well were plated, and DNA from 10 single colonies was analyzed for plasmid insert size. 3 out of
40 the 10 colonies showed an insert of about 2.1 kilobases in length, large enough to encode the IL-12 receptor. One of these clones was picked for further analysis.

Example 21

45 Characterization of IL-12 Receptor cDNAs

IL-12 receptor clone No. 5 was picked as described above and the plasmid DNA isolated. Gel isolated insert was sequenced on both strands using the ABI automated DNA sequencer in conjunction with a thermostable DNA polymerase and dye-labelled dideoxynucleotides as terminators.
50 Sequence alignments were run using the ALIGN program (M. O. Dayhoff et al. (1983) Meth. Enzymol. 91, 524) with the mutation data matrix, a break penalty of 6 and 100 random runs.

Cloned IL-12 receptor cDNAs were expressed in COS cells using either the DEAE dextran transfection or electroporation techniqu s (Mol cular Cloning Manual). Binding assays with labelled IL-12 or labelled 2-4E6 antibody wer run as described h r in abov und r anti human IL-12 rec ptor antibody. Th binding
55 data wer analyzed and Kd values w r calculated according to Scatchard, using th Ligand program discussed h r in above under anti human IL-12 receptor antibody. In vivo labelling (6 hours) of COS cells (3 x 10^5 cells per 35 mm diam t r tissu cultur dish) with ^{35}S Cyst in was performed 3 days aft r transfection as described (Molecular Cloning Manual). Cells w r washed in PBS and lysed in CHAPS lysis

buffer (10 mM CHAPS - 300 mM NaCl - 50 mM Tris-HCl pH 7.4 - 2 mg/ml Iodoac tamid - 0.17 mg/ml PMSF), precleared by incubation with protein G S pharose beads (50 µl packed beads per ml, Genex) and normal mous s rum (25 % final concentration) at 4°C overnight. Th beads wer spun out and labelled IL-12 rec ptor was specifically immunoprecipitated from th cl ared lysates by adding 5 µg of 2-4E6 antibody

5   per ml of sample. The antibody was diluted in PBS containing 1% bovine serum albumin and had been loaded onto 50 µl of packed beads for 2 - 3 hours at 4°C. Immunoprecipitation took place overnight at 4°C. The next day, the beads were washed 3-4 times in CHAPS lysis buffer before analysis on SDS-polyacrylamide gels as described (Molecular Cloning Manual).

10   Example 22

Lymphocyte Proliferation Assay

Lymphocyte proliferation assays to assess the ability of rat antisera to block cytokine-induced prolifera-
15   tion were performed as previously described (M.K. Gately, et al. (1992) Current Protocols in Immunology, vol. 1., J. E. Coligan, et al., eds., John Wiley & Sons, New York, NY, p. 6.16.1) with the following modifications. Aliquots of human PHA-activated PBMC (2×10^4 per well) and of diluted rat sera were mixed in the wells of a 96-well plate and incubated at 37°C for 30 min. The cytokines (IL-12, IL-2 or IL-7) were then added to the wells, and the cultures were incubated for 48 h at 37°C. Following this, ^{3}H-TdR was
20   added to each well, and the cultures were harvested after an additional 7 h at 37°C. All values are the means of triplicates.

Example 23

25   Flow Cytometry

The titers of anti-COS cell antibodies in the various rat antisera were assessed by flow cytometry as follows. Untransfected COS cells (10^6 cells/0.1 ml of Dulbecco's PBS containing 2% heat-inactivated FCS and 01 % sodium azide) were preincubated with 400 µg/ml normal rat IgG (Sigma, St. Louis, MO) for 15
30   min. on ice, and then with the indicated amount of rat serum for 30 min. on ice. The cells were washed and further incubated with 2 µg/ml FITC-conjugated F(ab')$_2$ mouse anti-rat IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) for 30 min. on ice. The cells were again washed and then analyzed by flow cytometry using a FACScan (Becton-Dickinson, Mountain View, CA).

35   Example 24

Inhibition of IL-12-induced Lymphoblast Proliferation by Anti-IL-12R Antiserum

To determine whether the cloned IL-12R subunit plays an essential role in an IL-12-induced biologic
40   response, we examined whether antiserum to the cloned IL-12R subunit could inhibit IL-12-induced proliferation of PHA-activated PBMC. This antiserum was produced by, immunizing rats with 2-4E6-transfected COS cells and thus contained anti-COS cell antibodies as well as antibodies to the (putative) IL-12R subunit. For use as a control, we screened several other available rat antisera which had been prepared previously by immunization of rats with COS cells expressing proteins unrelated to the IL-12R.
45   One such antiserum had been raised against COS cells transfected with human type II IL-1R and displayed a titer of anti-COS cell antibodies virtually identical to that of the anti-IL-12R antiserum (Figure 18A). We then compared the effects of the anti-IL-12R antiserum, the anti-IL-1R antiserum, and preimmune serum (from the rat used to prepare the anti-IL-12R) on lymphoblast proliferation induced by IL-12, IL-2, or IL-7. The concentrations of IL-12, IL-2, and IL-7 were 0.25 ng/ml, 1.25 ng/ml, and 0.4 ng/ml, respectively. These
50   concentrations were chosen because they resulted in similar levels of ^{3}H-TdR incorporation and were on the steep portion of the dose-response curves. In this experiment, the maximum levels of ^{3}H-TdR incorporation in the presence of saturating amounts of cytokine were 38,820, 111,303, and 89,541 cpm for IL-12, IL-2, and IL-7, r spectiv ly. Th l vel of ^{3}H-TdR incorporation in th absence of any add d cytokine is indicated by th horizontal dotted lin . Two experiments w r performed with ess ntially id ntical r sults, and on of
55   th s is illustrat d in Figur s 18B-D. Th anti-IL-12R antis rum caus d dose-d p ndent inhibition of IL-12-induced lymphoblast prolif ration but had no effect on proliferation induc d by IL-2 or IL-7. In contrast, neither the pr immune s rum nor th anti-IL-1R antiserum inhibited lymphoblast proliferation induced by any of th thr cytokines t sted. These r sults strongly suggest that the cloned IL-12R subunit plays an

20

Exampl 26

Analysis of IL-12 Rec ptor mRNA Expression

5   RNA blots were performed using polyA+ RNA from cells known to respond to IL-12: PHA-stimulated PBMC and the CD4+ T-cell line Kit225. Two RNA transcripts about 3 Kb and 2.3 Kb in size are apparent when blots are probed with the full-length cDNA (Figure 6, lanes 1-3). Both RNAs are induced from undetectable or very low levels when PBMC are activated by PHA for 3 days (compare lanes 1 and 2); Kit225 cells express both transcripts constitutively (lane 3). Analysis by phosphoimager shows the level of 10 the larger RNA to be about 3 to 5 fold higher than the level of the smaller RNA. Surprisingly, the smaller RNA does not hybridize to a probe derived from the cytoplasmic domain (lanes 4-6). This finding could indicate the presence of an RNA encoding i) a soluble IL-12 receptor protein, ii) a membrane bound IL-12 receptor devoid of a cytoplasmic region altogether or iii) an IL-12 receptor with a cytoplasmic sequence that is completely different from the ones present in clones 5 and 17. Elucidation of this question will have to 15 await the isolation of a cDNA clone derived from the smaller RNA transcript.

Example 27

Characterization of Recombinant IL-12 Receptor

20   IL-12 receptor cDNA (clone number 5) (SEQ ID NO:1) was electroporated into COS cells and equilibrium binding of labelled human and murine IL-12 to the cells was performed and analyzed as described (R. Chizzonite, et al., 1992, J. Immunol., 148, 3117). Results are shown in Figure 19. Human and murine IL-12 bind to recombinant IL-12 receptor (SEQ ID NO:2) with a single affinity (K_D) of 3.4 ± 1.3 nM 25 (n=7) and 2.1 ± 0.5 nM (n=4), respectively, which corresponds to the low affinity component of the functional IL-12 receptor on PHA-activated PBMC. After transformation by the method of Scatchard, the equilibrium binding data was best described by a single receptor site model as determined by the LIGAND program. The site numbers indicated in Figure 19 are calculated assuming that all cells are expressing receptors. IL-12 receptor protein expressed by clone number 17 (SEQ ID NO:3) gave similar results in 30 these binding assays. SEQ ID NO:3 would also have the same regions as SEQ ID NO:2.

    Metabolic labelling and immunoprecipitation of the IL-12 receptor subunit expressed in COS cells indicated its size to be about 100 KDa as determined by gel analysis under reducing conditions (Figure 5). To analyze the size of the receptor at the cell surface, affinity crosslinking studies were performed. Unless otherwise stated, characterization of the IL-12 receptor protein was done on SEQ ID NO:2. Crosslinking of 35 0.2 nM ^{125}I-labelled IL-12 to either transfected COS cells, PHA-activated PBMC or K6 cells gave rise to complexes that migrate at >200 KDa (Figure 17, lanes 1,3 and 4; arrow indicates uncrosslinked IL-12). Crosslinking at 2 nM ^{125}I-IL-12 (a concentration equivalent to the K_D) gave identical results (not shown). The size of a complex composed of one receptor subunit and one IL-12 ligand is expected to be about 175 KDa. However, Figure 17 shows that the 175 KDa complex is present only at very low levels, if at all. Since the 40 150 kDA Ig and the 200 KDa markers are not separated on the gel system used, the 175 KDa IL-12/IL-12 receptor complex is expected to comigrate with them. For comparison, lane 2 shows transfected COS cells crosslinked to labelled 2-4E6 antibody (arrowhead = uncrosslinked 2-4E6). Crosslinking labelled IL-12 to i) cells that do not bind IL-12 (e.g. Raji cells), ii) mock-transfected COS cells or iii) transfected COS cells in the presence of an excess of cold IL-12 did not yield any products (not shown). For Figure 17, labelled IL- 45 12 (0.2 nM) was bound and crosslinked with BS3 (0.4 mM) to transfected COS cells (lane 1), PHA-activated PBMC (lane 3) or K6 cells (lane 4). Labelled 2-4E6 antibody (0.5 nM) was bound and crosslinked with BS3 (0.4 mM) to transfected COS cells (lane 2). Anti-IL-12 receptor antibody 2-4E6 (lanes 5,7), anti-IL12 antibody 4D6 (lanes 9,11) and control antibody (lanes 6,8,10,12,) were used. Antibody 2-4E6 (lanes 13,15) and control antibody (lanes 14,16) were used.

50   Since crosslinking of labelled IL-12 to IL-12 receptor gave rise to products that are larger than what is expected for a complex of one receptor and one IL-12 ligand but whose size is difficult to estimate, cell surface labelling and immunoprecipitation experiments of transfected COS cells were performed. Samples wer analyzed under reducing and nonreducing conditions (Figur 17, lanes 5-12). Th results can be summariz d as follows: i) transfect d COS c lls xpress th IL-12 rec ptor subunit both as monom rs and 55 as a second, larger product that could b dim rs or oligomers. Both th s products ar pr sent at about equal l vels (lan 5); ii) the dim rization/oligom rization does not dep nd on IL-12 binding. If IL-12 is prebound to th cells, th resulting banding pattern does not chang (not shown); and iii) Th dimers/oligomers can b convert d to the monomers by reduction and must ther for be disulfide-bond d

(lan 7). Th data from th crosslinking and surfac labelling exp rim nts thus suggested that only the dimeric/oligomeric rec ptor subunit form binds IL-12 with the 3 nM affinity measured on transfected COS c lls. This possibility was further investigated as follows. Compl x s produc d by binding unlabelled IL-12 to ^{125}I-surfac labelled COS c lls and crosslinking with a cleavable crosslink r were immunoprecipitated by

5 an anti-IL-12 antibody and analyzed under non-reducing and reducing conditions (Figure 17, lanes 9-12). The anti-IL-12 antibody only precipitated a complex corresponding to IL-12 bound to the dimer/oligomer but not the monomer of the IL-12 receptor subunit (lane 9). Analysis of this complex under reducing conditions identified a labelled protein that co-migrated with the IL-12 receptor monomer (lane 11). Experiments with a murine CTLL cell transfectant stably expressing the IL-12 receptor subunit lend further support to our

10 findings. These cells express about 3000 to 5000 receptor subunits per cell, as measured by 2-4E6 antibody binding; however, the cells bind IL-12 very inefficiently, with an estimated Kd of 50 nM or greater (not shown). The results from surface labelling and immunoprecipitation experiments with the CTLL transfectants clearly indicate that they only express IL-12 receptor subunit monomers (Figure 17, lanes 13-16). Taken together, the data support the hypothesis that only the receptor subunit dimers/oligomers bind

15 IL-12 with the low affinity (3 nM) measured on transfected COS cells.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT:
        (A) NAME: F. HOFFMANN-LA ROCHE AG
        (B) STREET: Grenzacherstrasse 124
        (C) CITY: Basle
        (D) STATE: BS
        (E) COUNTRY: Switzerland
        (F) POSTAL CODE (ZIP): CH-4002
        (G) TELEPHONE: 061 - 688 51 08
        (H) TELEFAX: 061 - 688 13 95
        (I) TELEX: 962292/965542 hlr ch

    (ii) TITLE OF INVENTION: INTERLEUKIN-12 RECEPTOR

    (iii) NUMBER OF SEQUENCES: 3

    (iv) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: Apple Macintosh
        (C) OPERATING SYSTEM: System 7.1 (Mac)
        (D) SOFTWARE: Microsoft Word 5.0

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 2104 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA to mRNA

    (iii) HYPOTHETICAL: NO

    (vi) ORIGINAL SOURCE:
        (A) ORGANISM: Homo sapiens
        (G) CELL TYPE: human T-cells

    (vii) IMMEDIATE SOURCE:
        (A) LIBRARY: library 3 day PHA/pEF-BOS
        (B) CLONE: human interleukin-12 receptor clone #5

    (ix) FEATURE:
        (A) NAME/KEY: CDS
        (B) LOCATION: 65..2050

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GGTGGCTGAA CCTCGCAGGT GGCAGAGAGG CTCCCCTGGG GCTGTGGGGC TCTACGTGGA        60

TCCG ATG GAG CCG CTG GTG ACC TGG GTG GTC CCC CTC CTC TTC CTC TTC        109
     Met Glu Pro Leu Val Thr Trp Val Val Pro Leu Leu Phe Leu Phe
      1           5               10                  15

CTG CTG TCC AGG CAG GGC GCT GCC TGC AGA ACC AGT GAG TGC TGT TTT        157
Leu Leu Ser Arg Gln Gly Ala Ala Cys Arg Thr Ser Glu Cys Cys Phe
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CAG GAC CCG CCA TAT CCG GAT GCA GAC TCA GGC TCG GCC TCG GGC CCT          205
Gln Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro
            35              40              45

AGG GAC CTG AGA TGC TAT CGG ATA TCC AGT GAT CGT TAC GAG TGC TCC          253
Arg Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser
            50              55              60

TGG CAG TAT GAG GGT CCC ACA GCT GGG GTC AGC CAC TTC CTG CGG TGT          301
Trp Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys
    65              70              75

TGC CTT AGC TCC GGG CGC TGC TGC TAC TTC GCC GCC GGC TCA GCC ACC          349
Cys Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr
80              85              90              95

AGG CTG CAG TTC TCC GAC CAG GCT GGG GTG TCT GTG CTG TAC ACT GTC          397
Arg Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val
            100             105             110

ACA CTC TGG GTG GAA TCC TGG GCC AGG AAC CAG ACA GAG AAG TCT CCT          445
Thr Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro
            115             120             125

GAG GTG ACC CTG CAG CTC TAC AAC TCA GTT AAA TAT GAG CCT CCT CTG          493
Glu Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu
            130             135             140

GGA GAC ATC AAG GTG TCC AAG TTG GCC GGG CAG CTG CGT ATG GAG TGG          541
Gly Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp
    145             150             155

GAG ACC CCG GAT AAC CAG GTT GGT GCT GAG GTG CAG TTC CGG CAC CGG          589
Glu Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg
160             165             170             175

ACA CCC AGC AGC CCA TGG AAG TTG GGC GAC TGC GGA CCT CAG GAT GAT          637
Thr Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp
            180             185             190

GAT ACT GAG TCC TGC CTC TGC CCC CTG GAG ATG AAT GTG GCC CAG GAA          685
Asp Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu
            195             200             205

TTC CAG CTC CGA CGA CGG CAG CTG GGG AGC CAA GGA AGT TCC TGG AGC          733
Phe Gln Leu Arg Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser
            210             215             220

AAG TGG AGC AGC CCC GTG TGC GTT CCC CCT GAA AAC CCC CCA CAG CCT          781
Lys Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro
    225             230             235

CAG GTG AGA TTC TCG GTG GAG CAG CTG GGC CAG GAT GGG AGG AGG CGG          829
Gln Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg
240             245             250             255

CTG ACC CTG AAA GAG CAG CCA ACC CAG CTG GAG CTT CCA GAA GGC TGT          877
Leu Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys
            260             265             270
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CAA GGG CTG GCG CCT GGC ACG GAG GTC ACT TAC CGA CTA CAG CTC CAC          925
Gln Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His
            275                 280                 285

ATG CTG TCC TGC CCG TGT AAG GCC AAG GCC ACC AGG ACC CTG CAC CTG          973
Met Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu
            290                 295                 300

GGG AAG ATG CCC TAT CTC TCG GGT GCT GCC TAC AAC GTG GCT GTC ATC         1021
Gly Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile
        305                 310                 315

TCC TCG AAC CAA TTT GGT CCT GGC CTG AAC CAG ACG TGG CAC ATT CCT         1069
Ser Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro
320                 325                 330                 335

GCC GAC ACC CAC ACA GAA CCA GTG GCT CTG AAT ATC AGC GTC GGA ACC         1117
Ala Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr
                340                 345                 350

AAC GGG ACC ACC ATG TAT TGG CCA GCC CGG GCT CAG AGC ATG ACG TAT         1165
Asn Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr
            355                 360                 365

TGC ATT GAA TGG CAG CCT GTG GGC CAG GAC GGG GGC CTT GCC ACC TGC         1213
Cys Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys
            370                 375                 380

AGC CTG ACT GCG CCG CAA GAC CCG GAT CCG GCT GGA ATG GCA ACC TAC         1261
Ser Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr
        385                 390                 395

AGC TGG AGT CGA GAG TCT GGG GCA ATG GGG CAG GAA AAG TGT TAC TAC         1309
Ser Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr
400                 405                 410                 415

ATT ACC ATC TTT GCC TCT GCG CAC CCC GAG AAG CTC ACC TTG TGG TCT         1357
Ile Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser
                420                 425                 430

ACG GTC CTG TCC ACC TAC CAC TTT GGG GGC AAT GCC TCA GCA GCT GGG         1405
Thr Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly
            435                 440                 445

ACA CCG CAC CAC GTC TCG GTG AAG AAT CAT AGC TTG GAC TCT GTG TCT         1453
Thr Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser
            450                 455                 460

GTG GAC TGG GCA CCA TCC CTG CTG AGC ACC TGT CCC GGC GTC CTA AAG         1501
Val Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys
            465                 470                 475

GAG TAT GTT GTC CGC TGC CGA GAT GAA GAC AGC AAA CAG GTG TCA GAG         1549
Glu Tyr Val Val Arg Cys Arg Asp Glu Asp Ser Lys Gln Val Ser Glu
480                 485                 490                 495

CAT CCC GTG CAG CCC ACA GAG ACC CAA GTT ACC CTC AGT GGC CTG CGG         1597
His Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg
                500                 505                 510

GCT GGT GTA GCC TAC ACG GTG CAG GTG CGA GCA GAC ACA GCG TGG CTG         1645
Ala Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu
            515                 520                 525
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AGG GGT GTC TGG AGC CAG CCC CAG CGC TTC AGC ATC GAA GTG CAG GTT      1693
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TCT GAT TGG CTC ATC TTC TTC GCC TCC CTG GGG AGC TTC CTG AGC ATC      1741
Ser Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile
        545               550               555

CTT CTC GTG GGC GTC CTT GGC TAC CTT GGC CTG AAC AGG GCC GCA CGG      1789
Leu Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg
560               565               570               575

CAC CTG TGC CCG CCG CTG CCC ACA CCC TGT GCC AGC TCC GCC ATT GAG      1837
His Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu
                580               585               590

TTC CCT GGA GGG AAG GAG ACT TGG CAG TGG ATC AAC CCA GTG GAC TTC      1885
Phe Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe
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CAG GAA GAG GCA TCC CTG CAG GAG GCC CTG GTG GTA GAG ATG TCC TGG      1933
Gln Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp
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GAC AAA GGC GAG AGG ACT GAG CCT CTC GAG AAG ACA GAG CTA CCT GAG      1981
Asp Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu
        625               630               635

GGT GCC CCT GAG CTG GCC CTG GAT ACA GAG TTG TCC TTG GAG GAT GGA      2029
Gly Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly
640               645               650               655

GAC AGG TGC AAG GCC AAG ATG TGATCGTTGA GGCTCAGAGA GGGTGAGTGA        2080
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CTCGCCCGAG GCTACGTAGC CTTT                                          2104
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(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 662 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (ix) FEATURE:
        (A) NAME/KEY: Region
        (B) LOCATION: 1..23
        (D) OTHER INFORMATION: /note= "N-terminal signal peptide
            (1..20 or 23 or 24)"

    (ix) FEATURE:
        (A) NAME/KEY: Region
        (B) LOCATION: 541..570
        (D) OTHER INFORMATION: /note= "transmembrane region"

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    (B) LOCATION: 571..662
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    (B) LOCATION: 577..584
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    (B) LOCATION: 618..629
    (D) OTHER INFORMATION: /note= "conserved area of cytoplasmic tail
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    (B) LOCATION: 52..64
    (D) OTHER INFORMATION: /note= "sequence motif of cytokine
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    (B) LOCATION: 222..226
    (D) OTHER INFORMATION: /note= "cytokine receptor
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    (D) OTHER INFORMATION: /note= "N-linked glycosylation
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    (D) OTHER INFORMATION: /note= "N-linked glycosylation
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    (B) LOCATION: 442..444
    (D) OTHER INFORMATION: /note= "N-linked glycosylation
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(ix) FEATURE:
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      (B) LOCATION: 456..458
      (D) OTHER INFORMATION: /note= "N-linked glycosylation
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(ix) FEATURE:
      (A) NAME/KEY: Region
      (B) LOCATION: 24..540
      (D) OTHER INFORMATION: /note= "Extracellular region"


(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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            20                  25                  30

Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro Arg
            35                  40                  45

Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser Trp
        50                  55                  60

Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys Cys
65                  70                  75                  80

Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr Arg
                85                  90                  95

Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val Thr
            100                 105                 110

Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro Glu
        115                 120                 125

Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu Gly
    130                 135                 140

Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp Glu
145                 150                 155                 160

Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg Thr
                165                 170                 175

Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp Asp
            180                 185                 190

Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu Phe
            195                 200                 205

Gln Leu Arg Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser Lys
    210                 215                 220

Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro Gln
225                 230                 235                 240

Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg Leu
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Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys Gln
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Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu Gly
    290             295         300

Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile Ser
305             310         315             320

Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro Ala
            325             330         335

Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr Asn
            340             345         350

Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr Cys
            355             360         365

Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys Ser
    370             375         380

Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr Ser
385             390         395             400

Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr Ile
            405             410         415

Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser Thr
            420             425         430

Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly Thr
            435             440         445

Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser Val
    450             455         460

Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys Glu
    465             470         475             480

Tyr Val Val Arg Cys Arg Asp Glu Asp Ser Lys Gln Val Ser Glu His
            485             490         495

Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg Ala
            500             505         510

Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu Arg
            515             520         525

Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val Ser
    530             535         540

Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile Leu
545             550         555             560

Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg His
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Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu Phe
            580             585         590
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Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe Gln
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Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp Asp
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Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu Gly
625             630             635             640

Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly Asp
            645             650             655

Arg Cys Lys Ala Lys Met
            660
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(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 660 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro Arg
        35          40          45

Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser Trp
    50          55          60

Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys Cys
65          70          75          80

Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr Arg
            85          90          95

Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val Thr
            100         105         110

Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro Glu
        115         120             125

Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu Gly
    130         135         140

Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp Glu
145             150             155   .           160

Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg Thr
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Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp Asp
            180             185             190
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Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu Phe
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Gln Leu Arg Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser Lys
        210             215             220

Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro Gln
225             230             235             240

Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg Leu
            245             250             255

Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys Gln
        260             265             270

Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His Met
        275             280             285

Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu Gly
    290             295             300

Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile Ser
305             310             315             320

Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro Ala
            325             330             335

Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr Asn
            340             345             350

Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr Cys
        355             360             365

Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys Ser
    370             375             380

Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr Ser
385             390             395             400

Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr Ile
            405             410             415

Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser Thr
        420             425             430

Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly Thr
        435             440             445

Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser Val
    450             455             460

Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys Glu
465             470             475             480

Tyr Val Val Arg Cys Arg Asp Glu Asp Ser Lys Gln Val Ser Glu His
            485             490             495

Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg Ala
        500             505             510

Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu Arg
        515             520             525
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    Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val Ser
        530             535             540

    Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile Leu
    545             550             555             560

    Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg His
                565             570             575

    Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu Phe
                580             585             590

    Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe Gln
            595             600             605

    Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp Asp
        610             615             620

    Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu Gly
    625             630             635             640

    Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly Asp
                645             650             655

    Arg Cys Asp Arg
                660
```

Claims

1. A DNA molecule coding for a low affinity Interleukin-12 receptor or a functional derivative thereof which binds specifically to Interleukin-12.

2. A DNA molecule of claim 1 coding for a human low affinity Interleukin-12 receptor or a functional derivative thereof which binds specifically to Interleukin-12.

3. A DNA molecule of claims 1-2 having the sequence SEQ ID NO:1 or a degenerate variant thereof.

4. A DNA molecule of claims 1-3 which codes for the amino acid sequence SEQ ID NO:2 or SEQ ID NO:3 or portions thereof.

5. A DNA molecule of claims 1-4 which codes for a soluble, truncated Interleukin-12 receptor protein.

6. A vector comprising a DNA molecule as claimed in any of claims 1-5.

7. A host cell which has been transformed by a vector of claim 6.

8. A low affinity Interleukin-12 receptor protein or a functional derivative thereof which binds specifically to Interleukin-12.

9. A low affinity Interleukin-12 receptor protein encoded by a DNA molecule of claims 1-5.

10. A low affinity Interleukin-12 receptor protein of claims 8-9 wher in th Interl ukin receptor has a K_D of about 2 to about 10 nM, pr f rably a K_D of about 2 to about 5 nM.

11. A low affinity Interleukin-12 r c ptor protein of claims 8-10 having an apparent molecular weight of about 100 kDa d t rmined by gel lectrophor sis und r reducing conditions.

12. A low affinity Interleukin-12 r c ptor prot in of claims 8-11 which is a dim r or oligomer of th 100 kD r c ptor subunit.

13. A low affinity Int rl ukin-12 rec ptor prot in of claims 8-12 which corr sponds to the low affinity component of the functional Interleukin-12 receptor on PHA-activated PBMC.

14. An immunoglobulin capable of binding selectively to an Interleukin-12 receptor protein as defined in any one of claims 8-13.

15. An immunoglobulin as claimed in claim 14, which is in the form of an anti-sera composition or in the form of a monoclonal antibody.

16. The immunoglobulin of claim 14-15 which is capable of inhibiting the binding of Interleukin-12 to the Interleukin-12 receptor and is capable of neutralizing IL-12 bioactivity by binding to the IL-12 receptor.

17. The immunoglobulin of 14-15 which binds to the Interleukin-12 receptor but which is not capable of inhibiting the binding of Interleukin-12 to the Interleukin-12 receptor and is not capable of neutralizing Interleukin-12 bioactivity by binding to human IL-12 receptor.

18. A protein as claimed in any one of claims 8-13 for the binding or scavenge of Interleukin-12.

19. An immunoglobulin as claimed in any one of claims 14-17 for neutralising and/or inhibiting Interleukin-12 bioactivity.

20. A pharmaceutical composition comprising a low affinity Interleukin-12 receptor protein as defined in any one of claims 8-13 and a suitable diluent or carrier.

21. A pharmaceutical composition comprising an Interleukin-12 receptor protein as claimed in claim 20 containing one or more other cytokine receptor antagonists.

22. A pharmaceutical composition comprising an immunoglobulin as claimed in claim 14-17 and a suitable diluent or carrier.

23. A composition comprising human cells activated to express Interleukin-12 receptor bound with an immunoglobulin as claimed in claim 14-17, which immunoglobulin is labelled with a detectable label.

24. Use of an Interleukin- 12 receptor according to any one of claims 8-13 for the preparation of a pharmaceutical composition.

25. Use of an Interleukin-12 receptor according to claim 24 for the preparation of a pharmaceutical composition for treatment of diseases or conditions caused by an immune response to alloantigen and in the treatment of autoimmune dysfunction.

26. Use of an Interleukin-12 receptor protein as claimed in any one of claims 8-13 to bind or scavenge Interleukin-12.

27. Use of an immunoglobulin as claimed in claim 14-17 for the preparation of a pharmaceutical composition.

28. A process for the detection of an Interleukin-12 receptor which comprises isolating cells from a subject wich express the Interleukin-12 receptor, contatcing a sample of said cells with a detectable immunoglobulin capable of selectively binding to the Interleukin-12 receptor , incubating said cells under conditions which allow th detectabl immunoglobulin to bind to the Interleukin-12 receptor, and detecting th binding of said c lls to said immunoglobulin.

29. A process of claim 28 wh rein th immunoglobulin is covalently bound to a solid resin and/or said immunoglobulin is labelled with a d tectabl label.

30. A proc ss for th detection f soluble Int rl ukin-12 r c ptor which assay comprises capturing said receptor with an immunoglobulin capable of selectiv ly binding to the IL-12 receptor and carrying out a binding assay with lab lled Int rl ukin-12.

5 31. A method for determining an immune system abnormality in a subject which comprises determining the number of T cells, NK cells, or B cells in a sample from the subject, contacting the sample with an immunoglobulin capable of forming a complex with the human IL-12 receptor so as to form a cellular complex between the human IL-12 receptor and the immunoglobulin, determining the percentages of the T cells, NK cells, or B cells in the sample which have the human IL-12 receptor, comparing the
10 percentages of said cells with the percentages of such cells from a normal subject who does not have the immune system abnormality, a difference in the percentages of the cells so determined being indicative of the immune system abnormality.

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Fig. 1

```
          10         20         30         40         50         60         70
GGTGGCTGAA CCTCGCAGGT GGCAGAGAGG CTCCCCTGGG GCTGTGGGGC TCTACGTGGA TCCGATGGAG

          80         90        100        110        120        130        140
CCGCTGGTGA CCTGGGTGGT CCCCCTCCTC TTCCTCTTCC TGCTGTCCAG GCAGGGCGCT GCCTGCAGAA

         150        160        170        180        190        200        210
CCAGTGAGTG CTGTTTTCAG GACCCGCCAT ATCCGGATGC AGACTCAGGC TCGGCCTCGG GCCCTAGGGA

         220        230        240        250        260        270        280
CCTGAGATGC TATCGGATAT CCAGTGATCG TTACGAGTGC TCCTGGCAGT ATGAGGGTCC CACAGCTGGG

         290        300        310        320        330        340        350
GTCAGCCACT TCCTGCGGTG TTGCCTTAGC TCCGGGCGCT GCTGCTACTT CGCCGCCGGC TCAGCCACCA

         360        370        380        390        400        410        420
GGCTGCAGTT CTCCGACCAG GCTGGGGTGT CTGTGCTGTA CACTGTCACA CTCTGGGTGG AATCCTGGGC

         430        440        450        460        470        480        490
CAGGAACCAG ACAGAGAAGT CTCCTGAGGT GACCCTGCAG CTCTACAACT CAGTTAAATA TGAGCCTCCT

         500        510        520        530        540        550        560
CTGGGAGACA TCAAGGTGTC CAAGTTGGCC GGGCAGCTGC GTATGGAGTG GGAGACCCCG GATAACCAGG

         570        580        590        600        610        620        630
TTGGTGCTGA GGTGCAGTTC CGGCACCGGA CACCCAGCAG CCCATGGAAG TTGGGCGACT GCGGACCTCA

         640        650        660        670        680        690        700
GGATGATGAT ACTGAGTCCT GCCTCTGCCC CCTGGAGATG AATGTGGCCC AGGAATTCCA GCTCCGACGA

         710        720        730        740        750        760        770
CGGCAGCTGG GGAGCCAAGG AAGTTCCTGG AGCAAGTGGA GCAGCCCCGT GTGCGTTCCC CCTGAAAACC

         780        790        800        810        820        830        840
CCCCACAGCC TCAGGTGAGA TTCTCGGTGG AGCAGCTGGG CCAGGATGGG AGGAGGCGGC TGACCCTGAA

         850        860        870        880        890        900        910
AGAGCAGCCA ACCCAGCTGG AGCTTCCAGA AGGCTGTCAA GGGCTGGCGC CTGGCACGGA GGTCACTTAC

         920        930        940        950        960        970        980
CGACTACAGC TCCACATGCT GTCCTGCCCG TGTAAGGCCA AGGCCACCAG GACCCTGCAC CTGGGGAAGA

         990       1000       1010       1020       1030       1040       1050
TGCCCTATCT CTCGGGTGCT GCCTACAACG TGGCTGTCAT CTCCTCGAAC CAATTTGGTC CTGGCCTGAA

        1060       1070       1080       1090       1100       1110       1120
CCAGACGTGG CACATTCCTG CCGACACCCA CACAGAACCA GTGGCTCTGA ATATCAGCGT CGGAACCAAC

        1130       1140       1150       1160       1170       1180       1190
GGGACCACCA TGTATTGGCC AGCCCGGGCT CAGAGCATGA CGTATTGCAT TGAATGGCAG CCTGTGGGCC

        1200       1210       1220       1230       1240       1250       1260
AGGACGGGGG CCTTGCCACC TGCAGCCTGA CTGCGCCGCA AGACCCGGAT CCGGCTGGAA TGGCAACCTA

        1270       1280       1290       1300       1310       1320       1330
CAGCTGGAGT CGAGAGTCTG GGGCAATGGG GCAGGAAAAG TGTTACTACA TTACCATCTT TGCCTCTGCG
```

Fig. 1 Cont.

```
        1340       1350       1360       1370       1380       1390       1400
CACCCCGAGA AGCTCACCTT GTGGTCTACG GTCCTGTCCA CCTACCACTT TGGGGGCAAT GCCTCAGCAG

        1410       1420       1430       1440       1450       1460       1470
CTGGGACACC GCACCACGTC TCGGTGAAGA ATCATAGCTT GGACTCTGTG TCTGTGGACT GGGCACCATC

        1480       1490       1500       1510       1520       1530       1540
CCTGCTGAGC ACCTGTCCCG GCGTCCTAAA GGAGTATGTT GTCCGCTGCC GAGATGAAGA CAGCAAACAG

        1550       1560       1570       1580       1590       1600       1610
GTGTCAGAGC ATCCCGTGCA GCCCACAGAG ACCCAAGTTA CCCTCAGTGG CCTGCGGGCT GGTGTAGCCT

        1620       1630       1640       1650       1660       1670       1680
ACACGGTGCA GGTGCGAGCA GACACAGCGT GGCTGAGGGG TGTCTGGAGC CAGCCCCAGC GCTTCAGCAT

        1690       1700       1710       1720       1730       1740       1750
CGAAGTGCAG GTTTCTGATT GGCTCATCTT CTTCGCCTCC CTGGGGAGCT TCCTGAGCAT CCTTCTCGTG

        1760       1770       1780       1790       1800       1810       1820
GGCGTCCTTG GCTACCTTGG CCTGAACAGG GCCGCACGGC ACCTGTGCCC GCCGCTGCCC ACACCCTGTG

        1830       1840       1850       1860       1870       1880       1890
CCAGCTCCGC CATTGAGTTC CCTGGAGGGA AGGAGACTTG GCAGTGGATC AACCCAGTGG ACTTCCAGGA

        1900       1910       1920       1930       1940       1950       1960
AGAGGCATCC CTGCAGGAGG CCCTGGTGGT AGAGATGTCC TGGGACAAAG GCGAGAGGAC TGAGCCTCTC

        1970       1980       1990       2000       2010       2020       2030
GAGAAGACAG AGCTACCTGA GGGTGCCCCT GAGCTGGCCC TGGATACAGA GTTGTCCTTG GAGGATGGAG

        2040       2050       2060       2070       2080       2090       2100
ACAGGTGCAA GGCCAAGATG TGATCGTTGA GGCTCAGAGA GGGTGAGTGA CTCGCCCGAG GCTACGTAGC
```

Fig. 2

```
        10         20         30         40         50         60         70
MEPLVTWVVP LLFLFLLSRQ GAACRTSECC FQDPPYPDAD SGSASGPRDL RCYRISSDRY ECSWQYEGPT

        80         90        100        110        120        130        140
AGVSHFLRCC LSSGRCCYFA AGSATRLQFS DQAGVSVLYT VTLWVESWAR NQTEKSPEVT LQLYNSVKYE
                                                            ---

       150        160        170        180        190        200        210
PPLGDIKVSK LAGQLRMEWE TPDNQVGAEV QFRHRTPSSP WKLGDCGPQD DDTESCLCPL EMNVAQEFQL

       220        230        240        250        260        270        280
RRRQLGSQGS SWSKWSSPVC VPPENPPQPQ VRFSVEQLGQ DGRRRLTLKE QPTQLELPEG CQGLAPGTEV

       290        300        310        320        330        340        350
TYRLQLHMLS CPCKAKATRT LHLGKMPYLS GAAYNVAVIS SNQFGPGLNQ TWHIPADTHT EPVALNISVG
                                                          -- -              ---

       360        370        380        390        400        410        420
TNGTTMYWPA RAQSMTYCIE WQPVGQDGGL ATCSLTAPQD PDPAGMATYS WSRESGAMGQ EKCYYITIFA
---

       430        440        450        460        470        480        490
SAHPEKLTLW STVLSTYHFG GNASAAGTPH HVSVKNHSLD SVSVDWAPSL LSTCPGVLRE YVVRCRDEDS
                               ---        ---

       500        510        520        530        540        550        560
KQVSEHPVQP TETQVTLSGL RAGVAYTVQV RADTAWLRGV WSQPQRFSIE VQVSDWLIFF ASLGSFLSIL
                                                                 =================

       570        580        590        600        610        620        630
LVGVLGYLGL NRAARHLCPP LPTPCASSAI EFPGGKETWQ WINPVDFQEE ASLQEALVVE MSWDKGERTE
================    ---------                                    ===============

       640        650        660
PLEKTELPEG APELALDTEL SLEDGDRCKA KM
```

Fig. 3

```
IL-12R   GSASGI  _R  CY.RISSERY  ECSWQYEGPT  AGV.......  ..........
GP130    LPPEKPKNLS  CIVN.EGKKM  RCEWDGGRET  HLETNFTLKS  .......EWA
G-CSF-R  YPPAIPHNLS  CLMNLTTSSL  ICQWEPGPET  HLPTSFTLKS  FKSRGNCQTQ
LIF-R    YPPDTPQQLN  CETH.DLKEI  ICSWNPGRVT  ALVGP.RATS  YTLVESFSGK
CONS     .....P..L.  C.........  .C.W.....T  ..........  ..........

         SHFLRCC...  LSSGRCCYFA  AGSATRLQFS  DQAGVSVLY.  TVTLWVESWA
         THKFADCKAK  RDTPTSCTVD  YSTVYFVNI.  ..........  ..EVWVEAEN
         GDSILDCVPK  DGQSHCCIPR  KHLLLYQNM.  ..........  ..GIWVQAEN
         YVRLK.....  .........R  AEAPTNESYQ  LLFQMLPNQE  IYNFTLNAHN
         .h....C...  ......C...  ..........  ..........  ....vve...

         RNQTEKSPEV  TLQLYNSVKY  EPP......L  GDIKVSKLAG  QLRMEWETPD
         ALGKVTSDHI  NFDPVYKVKP  NPPHNLSVIN  SEE....LSS  ILKLTWTNPS
         ALGTSMSPQL  CLDPMDVVKL  EPPMLRTMDP  SPEAAPPQAG  CLQLCWEPWQ
         PLGRSQSTIL  .VNITEKVYP  HTPTSFKVKD  INSTA.....  .VKLSWHLPG
         ......S...  .......Vk.  .pP.......  ........l..  .l...W..p.

         NQVGAEVQF.  .RHRT.PSSP  WKLGDCGPQD  .DDTESCLCP  LEMNVAQEFQ
         IKSVIILKYN  IQYRTKDAST  W..SQIPPED  TASTRSSFTV  QDLKPFTEYV
         PGLHINQKCE  LRHKPQRGEA  SWALVGPLPL  EALQYELCGL  LPATAYTLQI
         NFAKINFLCE  IEIKKSNSVQ  EQRNVTIKGV  ENSSYLVALD  KLNP.YTLYT
         ..........  ...rt...s.  v......p.d  ...t.s....  .......e..

         LR.RRQLGSQ  GSSWSKWSSP  VC.VPPENPP  QPQVRFSVEQ  LGQDGRRRLT
         FRIRCMKEDG  KGYWSDWSEE  ASGITYEDRP  SKAPSFWYKI  DPSHTQGYRT
         RCIRWPLPGH  ...WSDWSPS  LELRTTERAF  TVRLDTWWRQ  RQLDP...RT
         FRIRCSTETF  W.KWSKWSNK  KQHLTTEASP  SKGPDTW.RE  WSSDG...KN
         .r.R......  ...WS.WS..  ......E..P  .....f....  .........t

         LKEQPTQLEL  PEGCQGLAPG  TEVTYRLQLH  MLSCPCKAKA  TRTLHLGKMP
         VQLVWKT..L  PPFEANGKIL  DYEVTLTRWK  SHLQNYTV..  ...NATKLTV
         VQLFWKP..V  PLEEDSGRIQ  GYVVS..WRP  SGQAGAILPL  CNTTELSCTF
         LIIYWKP..L  PINEANGKIL  SYNVSC...S  SDEETQSLSE  IPDPQHKAEI
         ..........l  P.........  ..........  ..........  ..........

         YLSGAAYNVA  VISSMQPGPG  LNQTWHIPAD  T...HTEPVA  LNISVGTMGT
         NLTNDRYLAT  LTVRNLVGKS  DAAVLTIPAC  DFQATHPVMD  LKAFPKDNNL
         HLPSEAQEVA  LVAYNSAGTS  RPTPVVFSES  R..GPALTRL  HAMARDPHSL
         RLDKNDYIIS  VVAKNSVGSS  PPSKIASMEI  P..NDDLKIE  QVVGMG.KGI
         .L....y...  ....M..G..  ......ipa.  ..........  l.......n..

         THYW.PARAQ  SHTYCIEWQP  VGQDGGLATC  SLTAPQDPDP  AGMATYSWSR
         WVEWTTPRES  VKKYILEWCV  LSDK...APC  .ITDMQQEDG  TVHRTYLR..
         WVGWEFPNPW  PQGYVIEWGL  GPPSASNSNK  TWRMEQNGRA  TG...FLLKE
         LLTWHYDPMN  TCDYVIKWCN  SSRS...EPC  LMDWRKVPSN  STETVI....
         ...W...r..  ...Y..eW..  .......a.c  ..t..q..d.  ....ty....

         ESGAMGQEKC  YYITIFASAH  PEKLTLNSTV  LSTYHFGGNA  SAAGTPHHVS
         ..GNLAESKC  YLITV.TPVY  ADGPG.SPES  IKAYL..KQA  PPSKGPT.VR
         NIRPFQ...L  YEIIVTPLYQ  DTM....GPS  QHVYAYSQEM  APSHAP.ELH
         ESDEFRPGIR  YNFFLYGCRN  QGYQLLRSMI  ..GY..IEEL  APIVAP.NFT
         ..g.....kc  Y.it......  ..........  ...Y......a  .....P..v.

         VKNHSLDSVS  VDWAPSLLST  CPGVLKEYV.  ..........  ..VRCRDEDS
         TKKVGKMEAV  LEWDQLPVDV  QNGFIRNYTI  FYRTIIGNET  AVNV......
         LKNIGKTWAQ  LEWVPEPPEL  GKSPLTHYTI  FWTMAQNQSF  SAIL......
         VEDTSADSIL  VEWEDIPVEE  LRGFLRGYLF  YPGKGERDTS  KDKVLESGRS
         .k........  ..W.......  ..g....Y..  ..........  ..........

         KQVSKHPVQP  TETQVTLSGL  RAGVAYTVQV  RADTAWLRGV  WSQPQ..RFS
         ........DS  SHTEYTLSSL  TSDTLYMVRM  AAYTDEGGKD  GPEFTFTTPK
         ........NA  SSRGFVLHGL  EPASLYHIHL  MAASQAGATN  STVLTLMTLT
         DIKVKNITDI  SQKTLRIADL  QGKTSYHLVL  RAYTDGGVGP  EKSHYVVTKE
         ..........  ..t..tls.L  .....Y.v..  .A.t......r  ..........

         IEVQVSDWLI  FFASLGSFLS  ILLVGVLGYL  GLNR..AARH  LCPPLPTPCA
         FAQGEIKAIV  VPVCLAFLLT  TLLGVLF..C  FNKRDLIKKH  IWPNVPDPSK
         PEGSELHIIL  GLPGLLLLLT  CLCGTAWLCC  SPNR...KNP  LNPSVPDPAH
         NSVGLIIAIL  IPVAVAVIVG  VVTS...ILC  YRKREWIKET  FYPDIPNPEN
         ..........  ....l...l.  .ll......  ...R.....h  ..P..P.P..

         SSAIEFPGGK  ETWQWINPVD  .PQEEASLQE  ALVVEM.SWD  KGERTEPLEK
         SHIAQWSPHT  PPRHNFNSKD  QMYSDGNFTD  VSVVEIEAND  KKPFPEDLKS
         SSLGSWVPTI  MEEDAPQLPG  L..GTPPITK  LTVLEEDEKK  PVPW.ESHNS
         CKALQFQKSV  CEGSS.ALKT  LEMNPCTPNN  VEVLETRSAF  PK.I.EDTEI
         s.........  ....n..d  ..........  ..VvE....d  k....E.l..

         TELPEGAPEL  ALDTELSLED  GDRCKAKM..  .
         LDLFK.KEKI  NTEGHSSGIG  GSSCMSSSRP  S
         SETCGLPTLV  QTYVLQGDPR  AVSTQPQSQS
         ISPVAKRPED  RSDAEPENHV  VVSYC
         ..l.......  ......s...  g..c......  .
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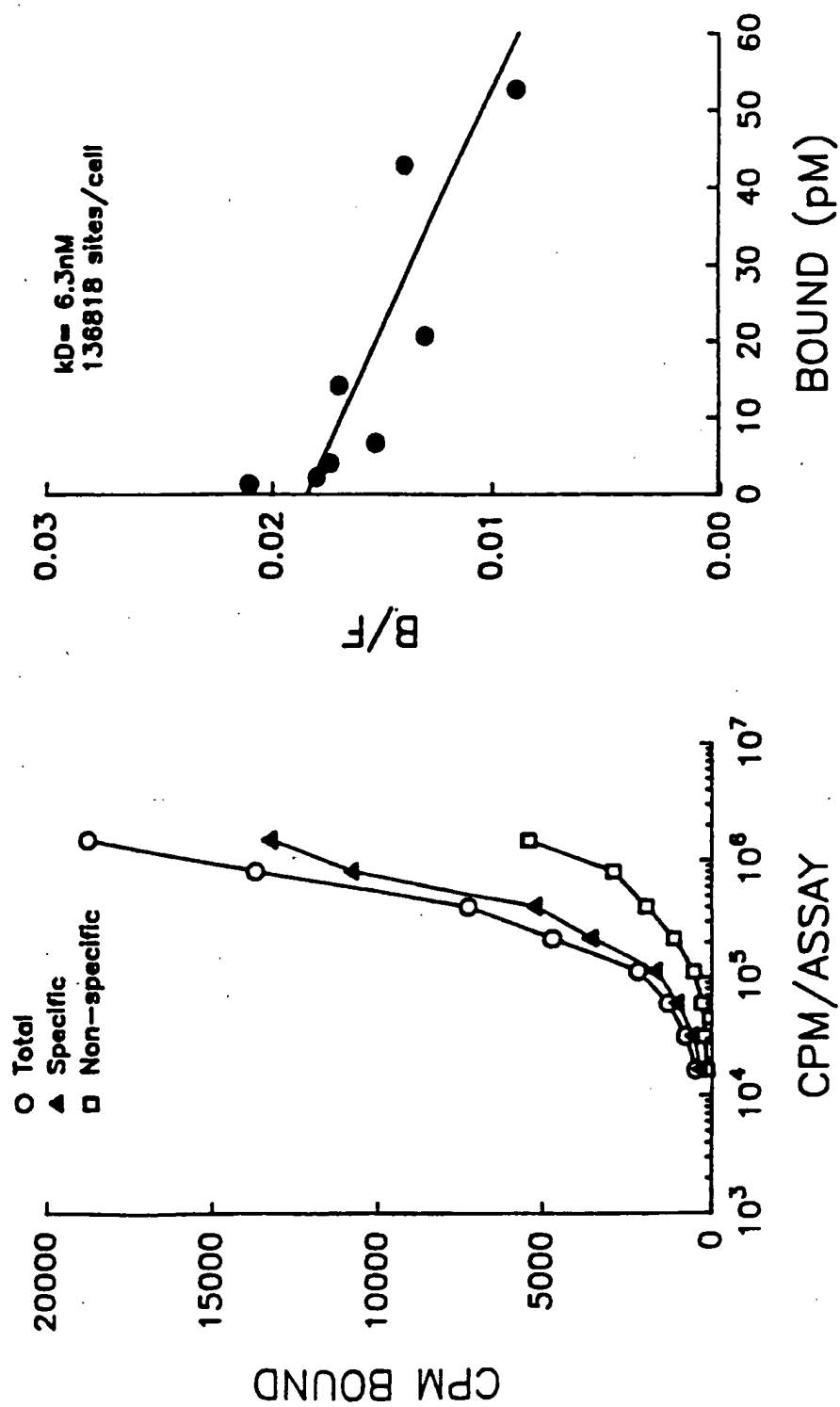
Fig. 4 a

COS(H12R) CELLS: [125]—I—HU—IL—12 BINDING

kD= 6.3nM
136818 sites/cell

Fig. 4 b

COS(H12R) CELLS: [125]-I-24E6 BINDING
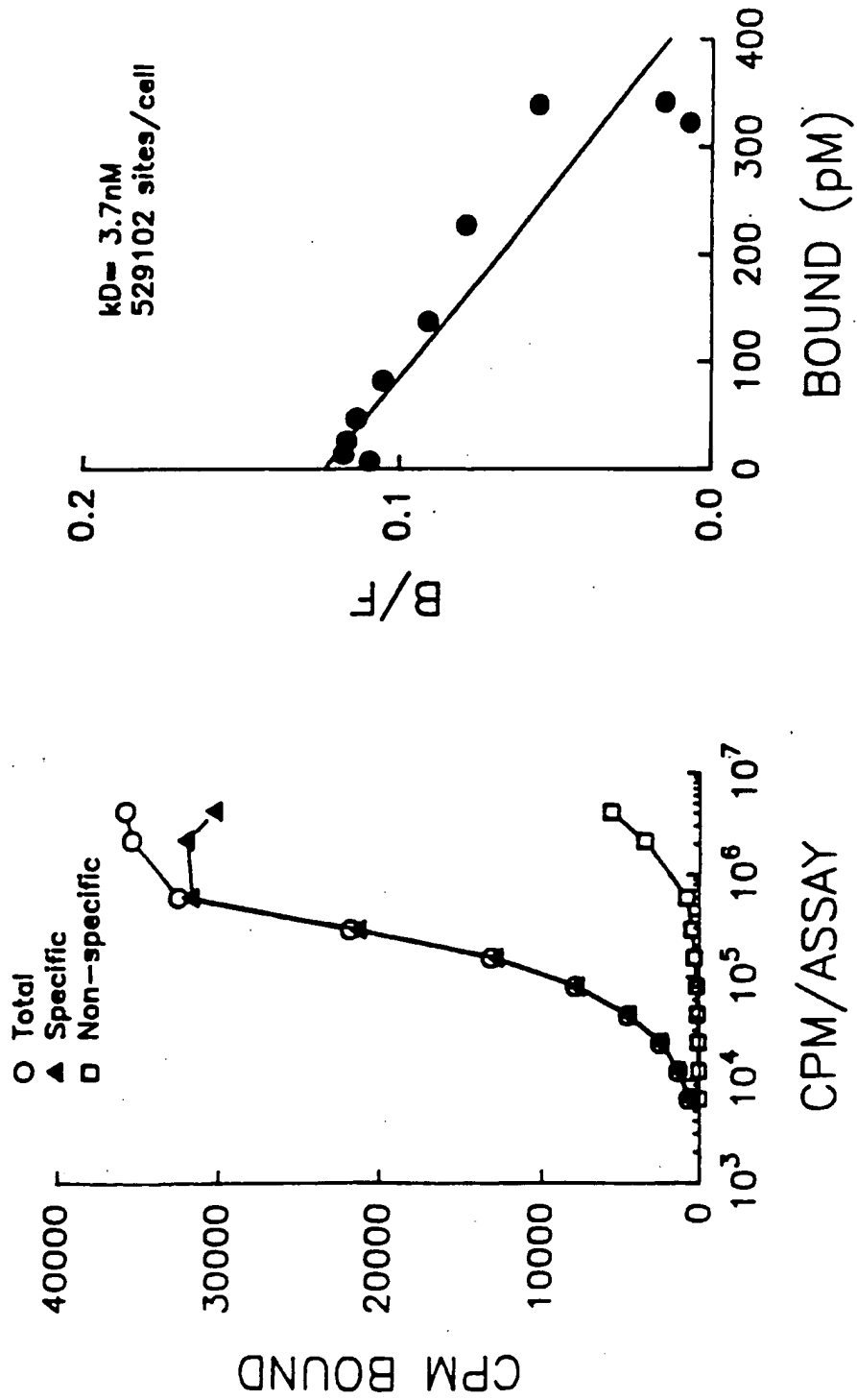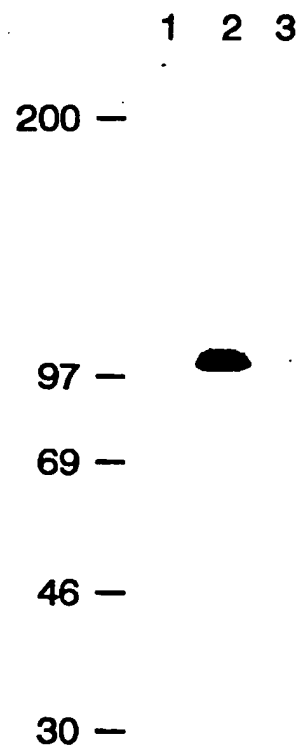
Fig. 5

1   2   3

200 —

97 —

69 —

46 —

30 —

Fig. 6
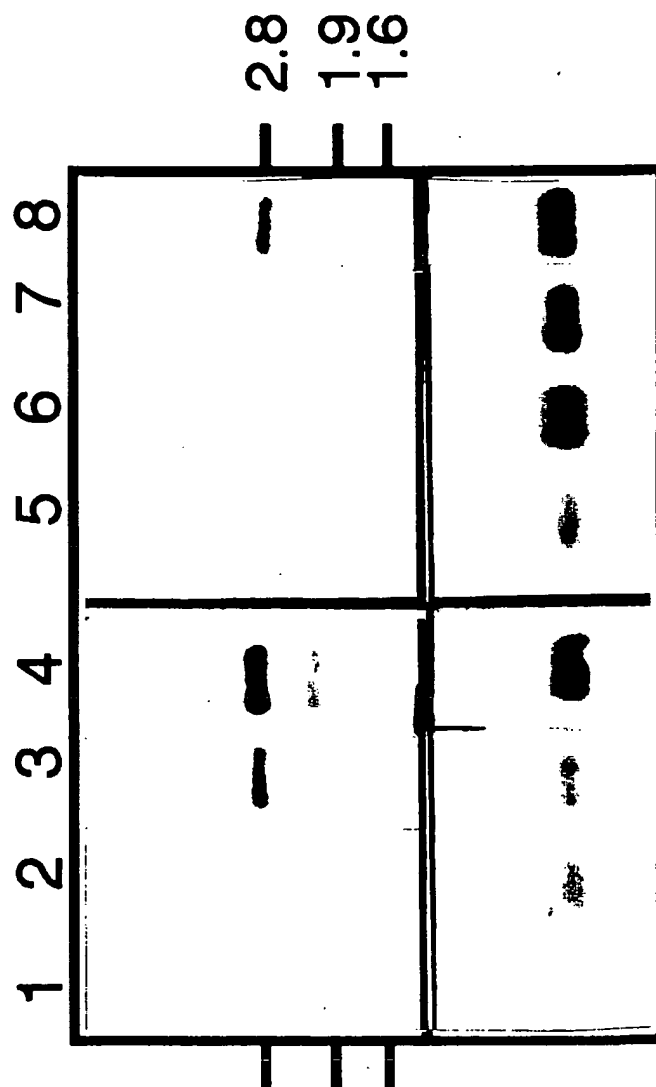
Fig. 7

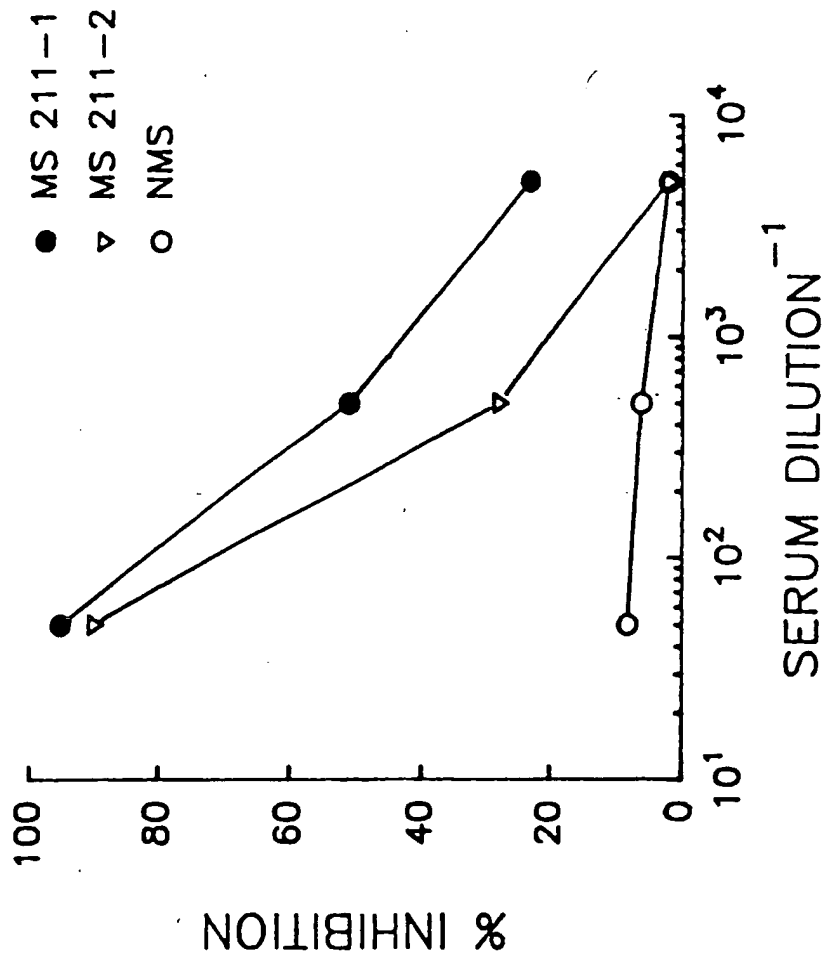INHIBITION OF [125]−I−IL−12 RECEPTOR BINDING BY MOUSE ANTI IL−12 RECEPTOR ANTISERUM

Fig. 8

Fig. 9

Fig. 10

Fig. 11

K6 CELLS: [125]—I—24E6 IgG BINDING

● Total
▼ Specific
▽ Non-specific

kD = 337 pM
4009 sites/cell
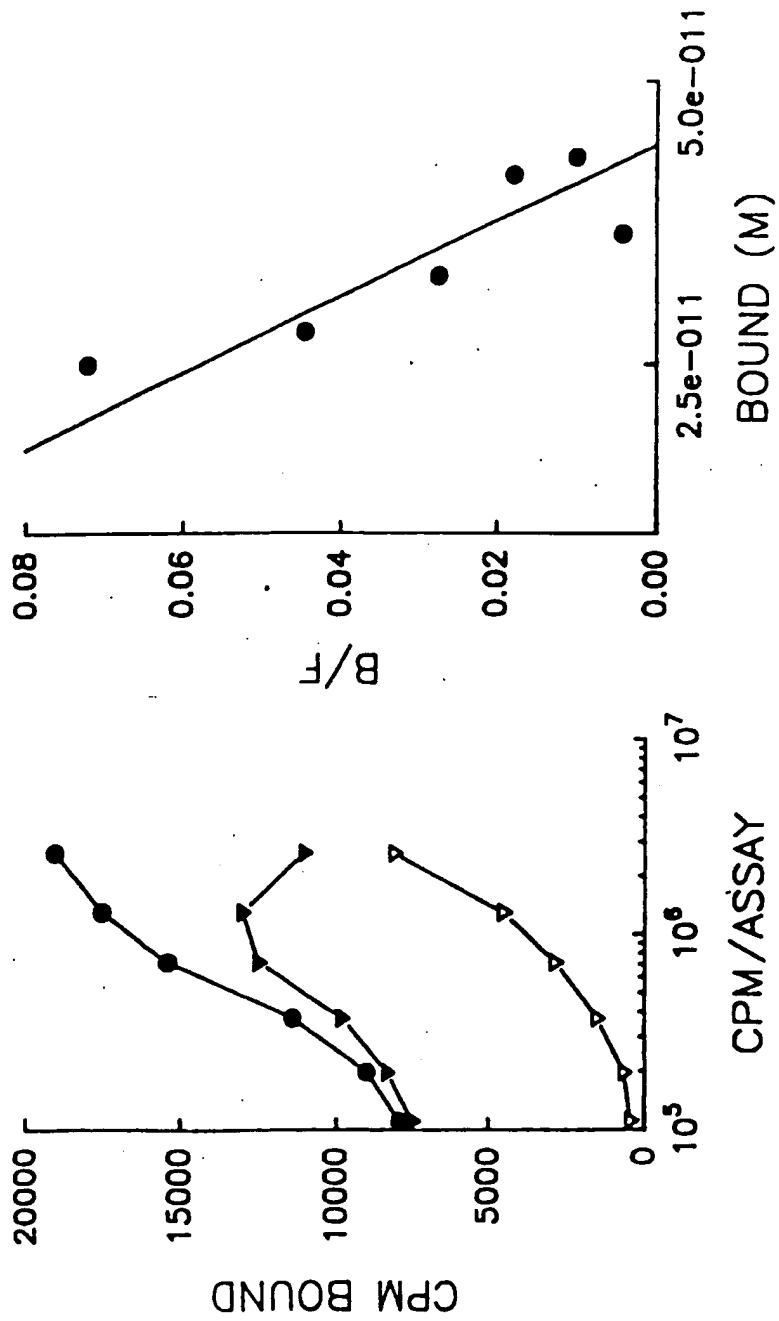
Fig. 12

125-I-24E6 COMPETITIVE BINDING TO K6 CELLS

o 24E6 IgG
▽ HUIL-12
● CONTROL IgG

IgG OR IL-12 CONCENTRATION (μg/ml)

Fig. 13

K6 CELLS: [125]-I-HUIL-12 (LSA) BINDING

o Total
▽ Specific
● Non-specific

kD = 16pM
707 sites/cell

kD = 57 pM
939 sites/cell

kD = 2.4nM
4036 sites/cell
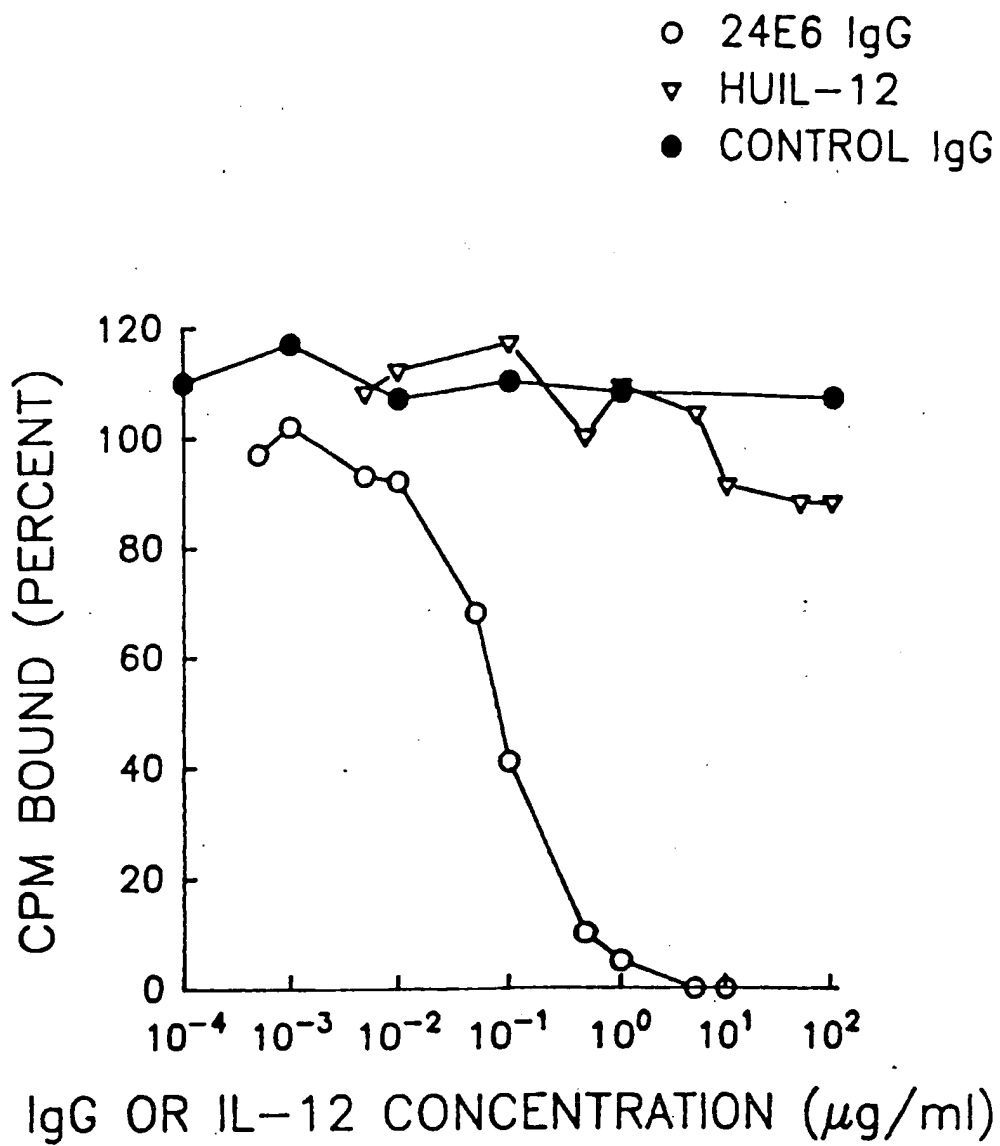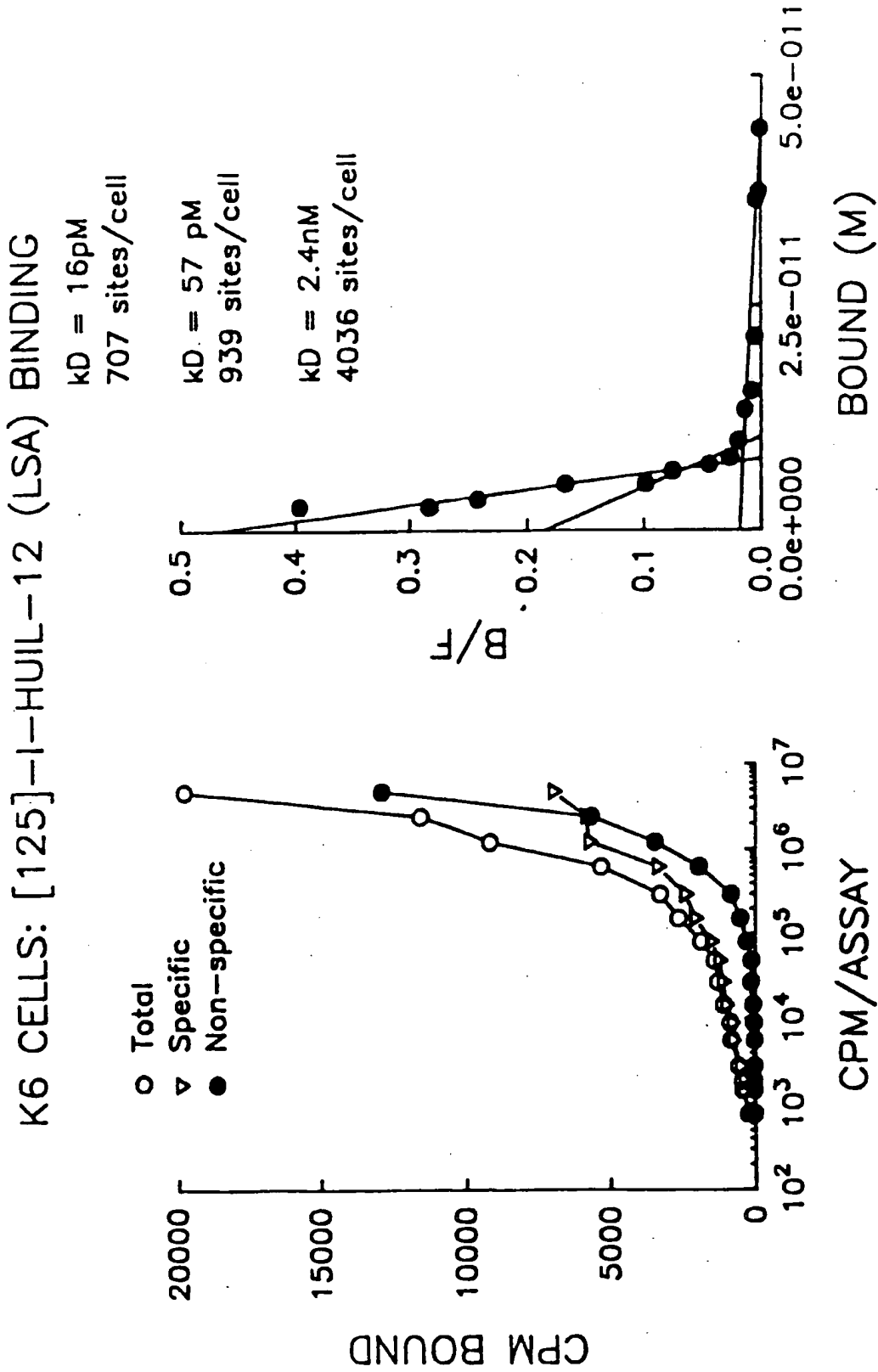
Fig. 14

[125]-I-HUIL-12 BINDING TO K6 SOLUBLE RECEPTOR

Fig. 15

Direct   Pre-bind IL-12
Solublization   and Solublize

2-4E6   4D6   Control IgG   STD   2-4E6   4D6   Control IgG

kDa

— 200

— 92.5

— 69.0

— 46.0

— 30.0
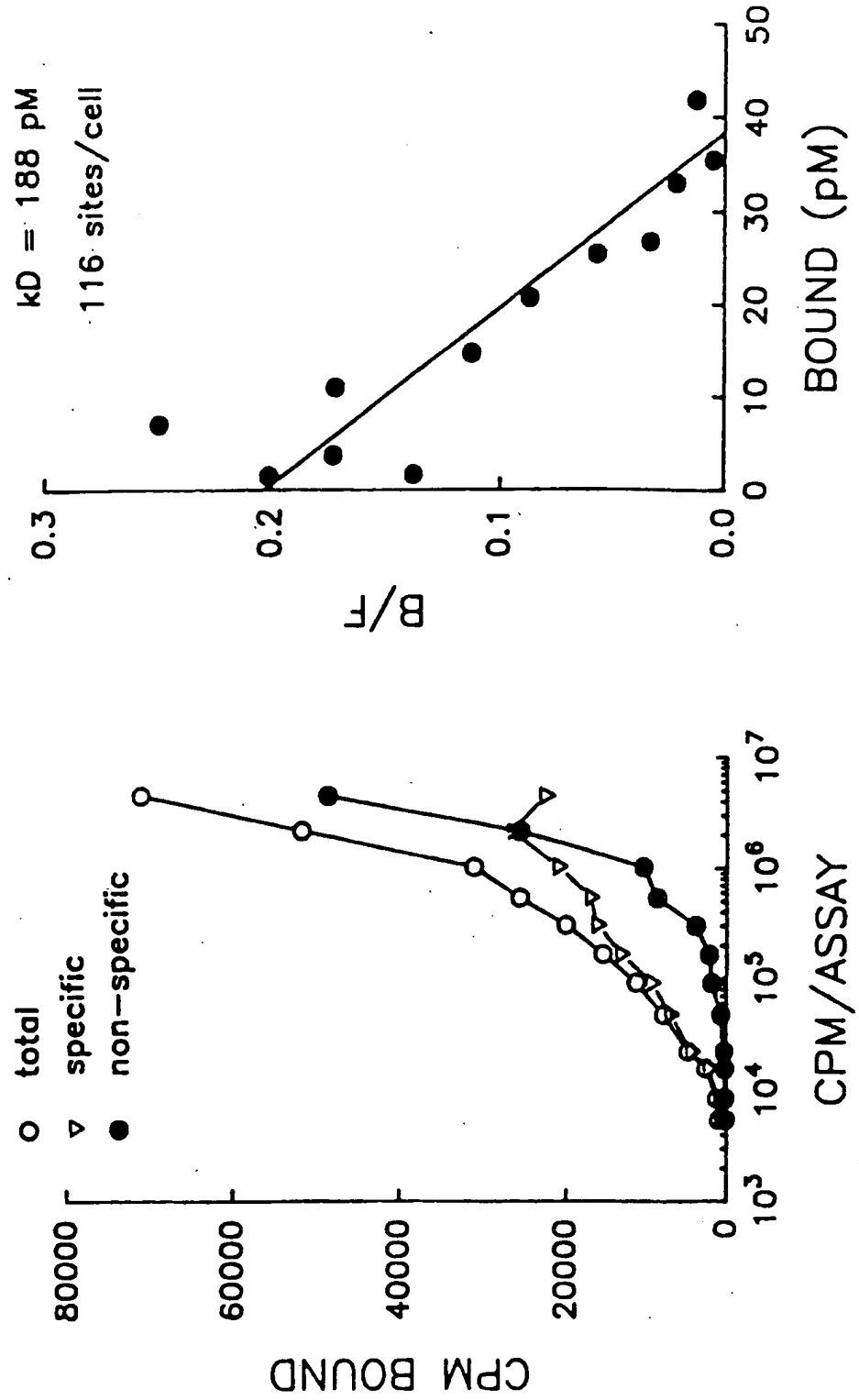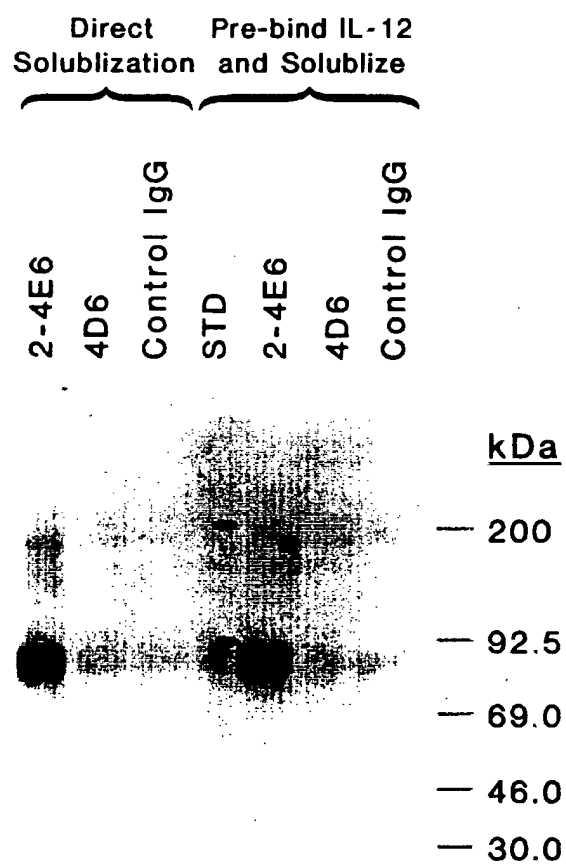
Fig. 16
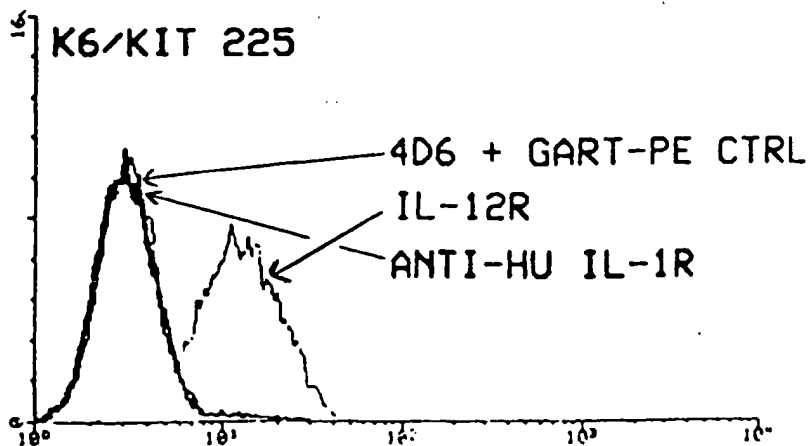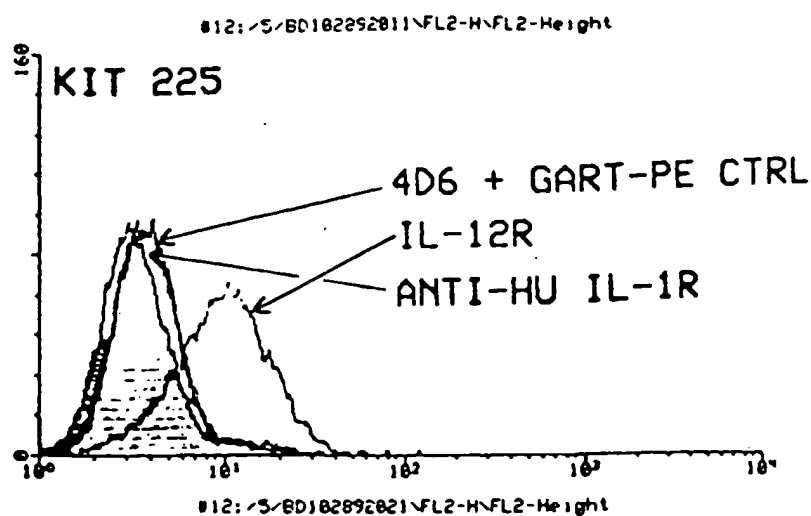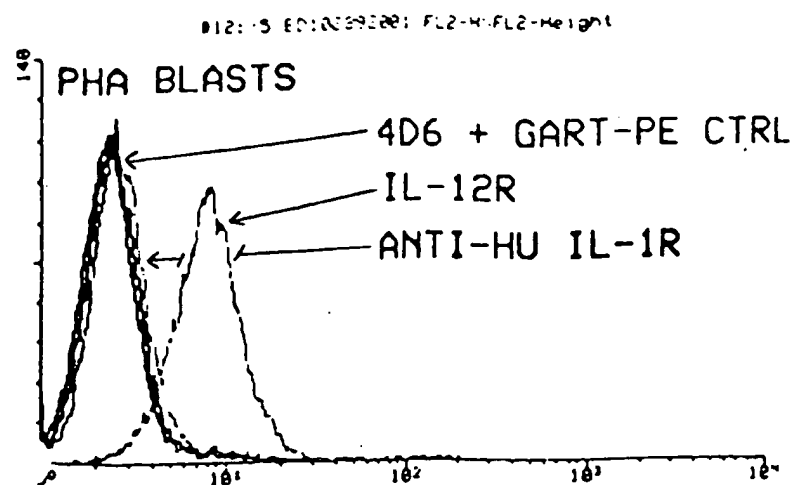
Fig. 17
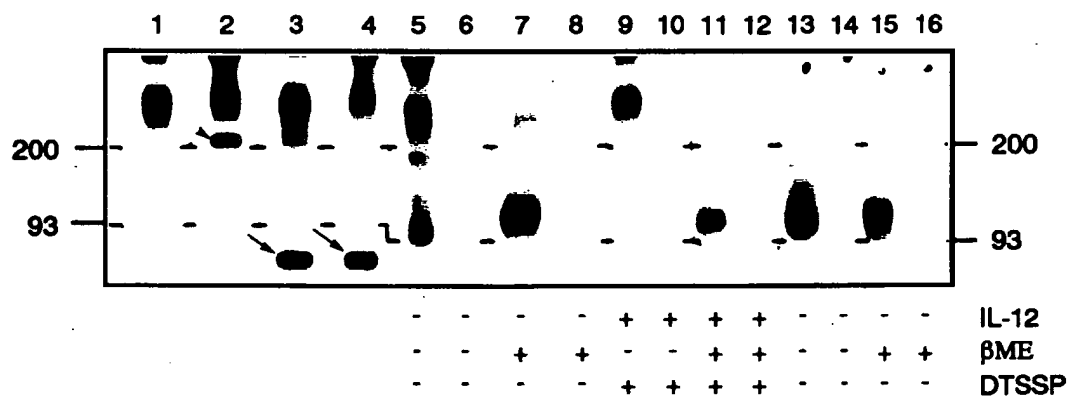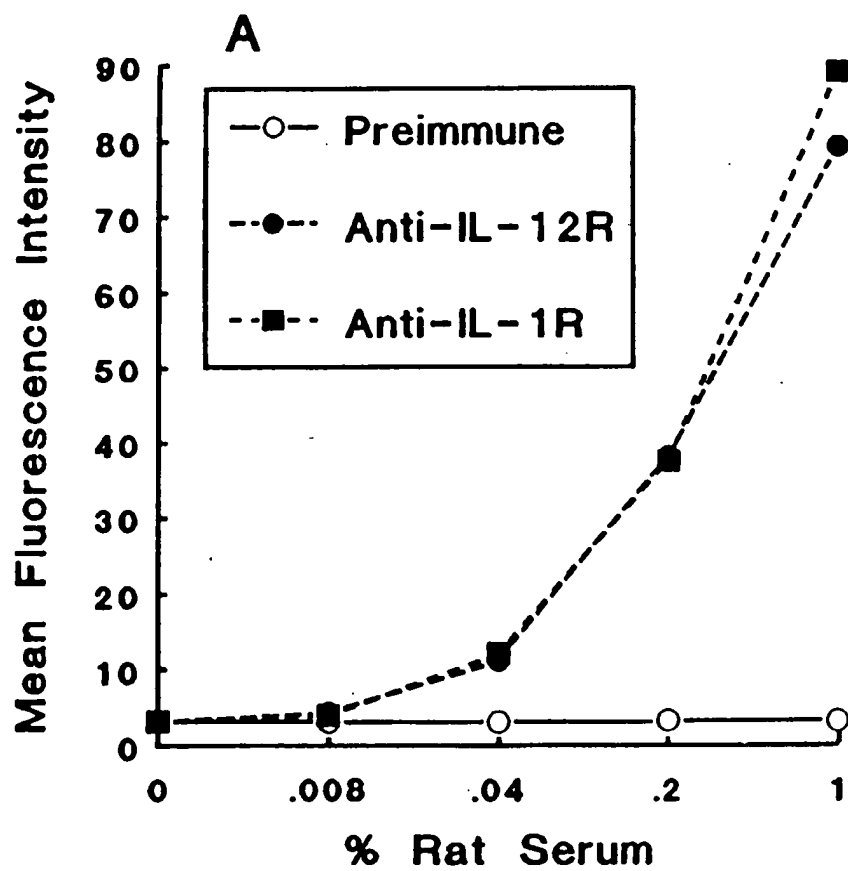
Fig. 18 a

Fig. 18 b

Fig. 18 c

Fig. 18 d

Fig. 19

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
P,X	JOURNAL OF IMMUNOLOGY., vol.153, no.1, 1 July 1994, BALTIMORE US pages 128 - 136 CHUA AO;CHIZZONITE R;DESAI BB;TRUITT TP;NUNES P;MINETTI LJ;WARRIER RR;PRESKY DH;LEVINE JF;GATELY MK;ET AL ; 'Expression cloning of a human IL-12 receptor component. A new member of the cytokine receptor superfamily with strong homology to gp130.' * the whole document * ---	1-31	C12N15/12 C07K14/715 C07K16/28 A61K38/17 A61K39/395 G01N33/68
X	JOURNAL OF IMMUNOLOGY., vol.150, no.8, May 1993, BALTIMORE US page 270A DESAI B;TRUITT T;HONASOGE S;WARRIER R;CHIZZONITE R;GATELY M; 'EXPRESSION OF FUNCTIONAL IL-12R ON A HUMAN IL-2-DEPENDENT T CELL LINE' & JOINT MEETING OF THE AMERICAN ASSOCIATION OF IMMUNOLOGISTS AND THE CLINICAL IMMUNOLOGY SOCIETY, DENVER, COLORADO, USA, MAY 21-25, 1993. ---	1-12,20, 21, 24-26, 28-31	**TECHNICAL FIELDS SEARCHED** (Int.Cl.6) C07K C12N A61K
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	5 December 1994	NAUCHE, S

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
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